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SIR JOHN SCOTT BURDON-SANDERSON, Bart. 1828—1905.

Sir John Scott Burdon-Sanderson was connected on both his father's and his mother's side with men of great distinction. His father, Richard Burdon-Sanderson, of West Jesmond and Otterburn Dene, was the son of Sir Thomas Burdon, several times Mayor of Newcastle. His grandmother was a sister of John Scott, famous afterwards as Lord Eldon, and of William Scott, who became Lord Stowell.

Richard Burdon went to Oxford for his University education, where he took first-class honours, obtained the Newdigate, and became a Fellow of Oriel College. He married the only daughter of Sir James Sanderson, Bart., M.P., who had been Lord Mayor of London; on his marriage he took the name of Sanderson. There were five children, two sons and three daughters; the oldest son, Richard Burdon-Sanderson, died from the effect of injuries received in the Abbott's Ripton railway accident of 1876; one of the daughters married Robert Haldane, of Cloanden, and two of her sons, the Right Hon. Richard Burdon Haldane, M.P., and Dr. John Scott Haldane, F.R.S., are well known, the former for his great public services, the latter for his contributions to science. John Scott Burdon-Sanderson, the fourth child, was born at North Jesmond, Northumberland, on December 21, 1828; the old Manor House had become unsafe owing to the undermining of the adjacent collieries, and the new house at West Jesmond was being erected for the future residence of the family.

In common with many other distinguished men of that period he was not sent to school, but was educated at home by tutors under the immediate supervision of his father. This home at West Jesmond, in the North Country, with its surrounding of swelling moors and rocky streams, fostered in the boy that love of nature and appreciation of natural objects which was to lead him into the biological side of natural science. He retained to the end of his life the impression of these early years, and always showed a special delight in wide space, the swell of hills stretching out to meet the sky, and all that airy freedom which is so indelibly bound up with moorland scenery. Shooting, fishing, and riding were the natural recreations of such a boyhood, but probably the greatest joy was experienced in scouring the stretches of wild country on horseback or on foot, often in the company of his father's huntsman, a most intelligent man, who knew every bird that flew. In later years there were few things which he found so exhilarating as a tramp over the heather, the enjoyment of which became much enhanced when he had developed his scientific knowledge. As a boy he delighted in the observation of wild animals and in the discovery of wild flowers; later he became a keen field botanist, experiencing all the satisfaction of the systematist, who, by naming a wild flower, places it into its proper place in the scale of plant life, experiencing also that peculiar pleasure which the field botanist knows when he comes upon a rare wild flower or one growing in unsuspected localities. In such surroundings were slowly forged the links which were to bind him to the study of living phenomena, and make him regard both pathological and physiological phenomena from a biological standpoint. There is little doubt that, with his inclinations, temperament and powers of observation, he possessed gifts which might have made him a great naturalist.

His father may have recognised the significance of these features of his son's character, as he undoubtedly recognised his ability, but the fame of the boy's great-uncles, Lord Eldon and Lord Stowell, inclined him to believe that a legal training would give the lad the best opportunity for their display.

An event, however, occurred which fixed the boy's own ideas as to his future. Dr. Mortimer Glover, of Newcastle, came to Otterburn to attend the elder brother, who was dangerously ill. Dr. Glover was an exceedingly able man, with a vivid sense of the great possibilities of his profession; he was at the time occupied in the experimental investigation of chloroform, and continued this work in his spare time at Otterburn. There is little doubt that he recognised young Burdon-Sanderson's intelligent interest in biological subjects, and that this friendship suggested to the boy the career of his life.

When Mr. Burdon-Sanderson discovered that his son's desire was to enter the medical profession, he wisely gave up his own views as to his future, and sent him in 1847 to Edinburgh, where he remained for about four years, taking the degree of M.D. in 1851, and obtaining the Gold Medal. Edinburgh was at this time the chief medical school in the United Kingdom, and the teaching staff comprised men of very great distinction; two of these seem to have exercised a particular influence upon young Burdon-Sanderson, Goodsir, then Professor of Anatomy, and Hughes Bennett, the Professor of Physiology; this was especially the case in regard to Bennett, who turned his thoughts and efforts towards the scientific and experimental side of medical science. It is somewhat remarkable that his earliest scientific investigations should have been related to subjects which were to employ for many years his mature powers.

In the Journal of the Royal Medical Society (a gathering of Edinburgh students for the discussion of scientific subjects) an entry occurs which states that, in 1850, a dissertation was read by John Scott Sanderson, of Newcastle, on "Vegetable Irritability." In the Edinburgh Monthly Journal of Medicine of 1851 there is an extensive criticism by John Scott Sanderson of the views held as to the metamorphosis of the red blood corpuscles; the criticism is founded on numerous experimental observations made by the author upon the blood of the spleen and other organs; from the title page of this paper, it seems that he was at this time Vice-President of the Physiological Society of Edinburgh.

It thus appears that, at the age of twenty-three or twenty-four, he had grasped some of those fundamental principles which were to be displayed in all his later scientific work. He recognised the importance of studying living processes, and the fundamental nature of such characteristics of these processes as are general in their distribution; for instance, excitability or irritability; he recognised also the paramount necessity of experimental investigation for the determination of a physiological problem. A further notable characteristic of his mental attitude towards biological and medical science is displayed by the step which he took when the Edinburgh curriculum had been completed. He appears to have realised early in his medical career that, since the study of living processes, whether normal or abnormal, was a study of their chemical and physical aspects, it was essential to possess an adequate knowledge of the methods used in chemistry.

The completion of medical education by a visit to a Continental medical school was in vogue at this time, and so Burdon-Sanderson went in the autumn of 1851 to Paris, with the definite intention of spending a considerable part of his time in working practically at organic chemistry under Gerhardt. In a little daily journal he dotted down brief indications as to the main occupations of each day; the diary is somewhat irregular, but is of much interest, since it affords a glimpse of the nature of his work.

In Paris he placed himself under Gerhardt, but soon began systematic work of a practical kind in the laboratory of Wurtz. He devoted himself especially to the study of the organic compounds which are found in animal tissues, and commenced a research into the constitution of such substances as could be extracted from muscle, particularly kreatine.

In the diary there are brief references to chemical work on a large variety of compounds, including kreatine, uric acid, urea, hippuric acid, benzoic acid, leucine, glycin, bile compounds, choleate of soda, stearic acid, elaic acid, oleic acid, lactic acid, and pulmonic acid, this last having a query annexed to it.

On January 16, 1852, there is a note to the effect that he read a paper at the Paris Medical Society "which was not understood." It was a busy winter; time was found for attending the embryological lectures given by Coste, for visiting once or twice a week some of the Paris hospitals, and for witnessing the experimental demonstrations given by Claude Bernard. Several of those who had been his fellow students at Edinburgh were in Paris at this time; among these the names of Pavy and Marcet frequently occur in the little diary. It appears that he joined these two friends in attending the experimental demonstrations and lectures given by Claude Bernard, and seems to have immediately fallen under the magnetic spell cast by the intellectual personality of this great physiologist. His veneration for Bernard lasted throughout his life, and in later years he used to say, pointing to the bust which stood upon the shelf above his study table, that Bernard was the most inspiring teacher, the most profound scientific thinker, and the most remarkable experimental physiologist that he had ever known. On March 1, 1852, there is the following interesting entry in the diary: "Went with Marcet to Bernard's, who showed us much attention;" followed the next day by, "Went with Harley and Wason to Bernard, who introduced us to Majendie ! !"; on March 13, 1851, occurs the entry "Was with Bernard performing several experiments." Among other entries scattered along the diary are the following : "Performed operation for obtaining pancreatic juice on a rabbit; did not succeed in introducing the cannula"; "injected a pigeon"; "operated unsuccessfully on a dog for pancreatic juice;" "operated successfully on pancreatic duct," etc. A more elaborate note-book of this date in his handwriting gives a synopsis of Bernard's experimental lectures, and treats some subjects in much detail, notably, the *rôle* and nature of glycogen and the phenomena of diabetes. He was in Paris during the exciting period of the *Coup d'Etat,* December 2, 1851; in 1852 he came back to England for Easter, but returned to Paris for May and June, mainly in order to finish some of his chemical work. He had now decided to settle in London, this resolve being largely determined by his approaching marriage with Ghetal Herschell, the daughter of the Rev. R. H. Herschell, and sister of Farrer Herschell, who subsequently became Lord Chancellor. In this union, which took place in 1853, he found, in the highest sense of the word, the helpmeet for him; all who knew Sir John and Lady Burdon-Sanderson are well aware of the peculiar strength of the ties which bound them, whilst those who have enjoyed the privilege of more intimate friendship realise that the great powers of the husband were fostered by the congenial atmosphere which the devoted care and loving sympathy of the wife ensured. In the serenity of true domestic felicity, a happiness which was to last without a check for over fifty years, he started in London as a practising physician. His scientific bent inclined him to take some scientific post if it should be offered him, and, in recognition of his extensive knowledge and undoubted ability, such offers soon occurred. He was in 1854 appointed Medical Registrar to St. Mary's Hospital, whilst a little later he was made Lecturer in Botany, and finally Lecturer in Medical Jurisprudence at the medical school attached to this institution. In 1854 he contributed, among other things, a masterly article on "Vegetable Reproduction" to Todd's Cyclopædia of Anatomy and Physiology, thus showing that his botanical interests remained as strong as ever.

In 1856 there occurred an event which may be considered as the first and greatest landmark in his scientific career, for it was the event which determined the specific character of his life's work; in this year he was appointed Medical Officer of Health for the extensive district of Paddington. That a post of such responsibility should be offered to a man under thirty years of age, and within three years of his arrival in London, is striking evidence of the high opinion which had been formed of his powers; these were now to be successively focussed on those branches of medical science in which he was to achieve acknowledged eminence. From practical sanitation he rapidly became an authority upon the scientific aspects of hygiene, and this study led by a natural extension to wider aspects of pathology, and ultimately back to physiology.

He retained his Paddington appointment for eleven years, one notable incident, the cholera epidemic of 1865–66, occurring towards the end of his period of office. His rare abilities and scientific acumen were soon recognised by the late Sir John Simon (at that time Mr. Simon), the Medical Officer of the Privy Council, and in 1860 he was appointed an Inspector in the Medical Department of the Council. In the discharge of these duties he sent in many sanitary reports, which were so valued that, although he ceased to be an acting inspector in 1866, he was frequently asked to undertake scientific investigations for the Government authorities, and continued to contribute to the annual reports of the Privy Council and Local Government Board for a further period of ten years. His contributions contain the results of experimental investigations on a number of scientific problems, which have subsequently become of extreme importance in connection with the prevention and etiology of infectious and other diseases. Sir John Simon, in his "Review of English Sanitary Institutions," gives some details which display the scope of Burdon-Sanderson's early work in practical sanitation, but a true appreciation of the debt which civilisation owes to Burdon-Sanderson can only be derived from a perusal of the reports themselves. It appears that from 1860 to 1864 he co-operated with others in collecting reliable information as to the utility of vaccination, and it was largely on the strength of this mass of information that a Bill to amend the Vaccination Law was laid before the Legislature, and that the important sanitary statute relating to this matter was passed in 1866. But the mental attitude of Burdon-Sanderson towards the problems presented by hygiene was one which impelled him to undertake a more profound experimental study of morbid processes, the subject being treated along chemical and physical lines without losing sight of the essential biological setting. In 1865 he commenced for the Medical Department of the Privy Council a laborious investigation of the chemical aspects of infective processes, and in particular of those which were associated with the presence of contagious diseases in animals. The special portion of this work with which his name will be always identified, is that devoted to the study of cattle plague, undertaken towards the close of 1865 for the Royal Commission appointed to investigate this very important matter. He ascertained that the true contagium could be separated by physico-chemical means from the animal tissues which contained it, and that the extracts of these tissues lost, in consequence of such separation, their infective power. This work brought the experimental study of contagious diseases in this country into line with the work of Pasteur upon fermentation and putrefaction. Another suggestive feature of his work at this time is furnished in the reports which he produced dealing with the subject of tuberculosis. The researches described in these reports are models of exactitude, and the descriptions of the way in which chronic tuberculous infection invades the organism is in all essentials that which is still put forth. At this period there was grave doubt as to the causative factor of tuberculosis, and even the possibility that the origin of the infection was bacterial in its nature was scarcely recognised by pathologists. In 1869 Sanderson's mental attitude as to the causative factors of contagious diseases was one which distinctly foreshadowed the views held at the present day. Alluding to the difficulties which were brought forward, he states explicitly that, as regards the most important of them, they disappear "if we suppose that the contagious process is connected with the unfolding of organic forms." At that time stress was laid upon chemical aspects of these pathological processes irrespective of their biological significance; Burdon-Sanderson, however, recognised the profound importance of these hypothetical micro-organisms. Thus, he states, with regard to such processes, that "it is difficult to see how the phenomena could be accounted for by their chemical properties, whereas there is good and scientific ground for anticipating that the solution of the problem will some day be attained by the investigation of the morphological phenomena which attend the infective process." A characteristic expression of his belief in the experimental method, as the instrument for furthering our knowledge of the subject, occurs in the sentence, "I am strongly impressed with the conviction that, if the investigation is to be successful, it must be experimental in every stage of its progress." In this, as in general pathology and physiology, he took up the attitude that the experimental study of the processes, normal and abnormal, indicated the sole course which could be steered, if the navigator would avoid shipwreck, upon the numerous rocks and shoals which beset this difficult and little known region.

The extent to which the value of this pathological work was recognised by his contemporaries is shown by the appreciative language of eminent scientific men. Thus, in 1870, Huxley drew attention to it in the remarkable address entitled "Biogenesis and Abiogenesis," which he delivered as President of the British Association. In this he pointed out that the report of Mr. Simon, the Medical Officer to the Privy Council, contained in an appendix a most valuable essay "On the Intimate Pathology of Contagion," by Dr. Burdon-Sanderson, "which," he went on to say, "is one of the clearest, most comprehensive, and well-reasoned discussions of a great question which has come under my notice for a long time."

In 1873 Burdon-Sanderson appears to have been firmly convinced that living forms were in reality the causal infective agents, and, as regards these forms, he stoutly opposed those who advocated the theory of spontaneous generation. At this time he was Professor Superintendent of the Brown Institute, a post which he held for seven years, from 1871 to 1878. One of the most suggestive of the many fruitful ideas which abound in this prolonged inquiry into infectious diseases occurs in this period, when, in concluding a report upon the nature of anthrax, he brought forward experimental evidence showing that the passage of anthrax virus derived from the ox through the bodies of guinea-pigs diminished its virulence for the bovine species. In concluding a report on this and allied subjects, he said: "The question whether this fact, like the analogous one of the mitigation of human small-pox by transmission, can be directly applied to a practical purpose, I leave to be determined by future inquiry." Three years afterwards Pasteur elaborated his system of protective inoculation against anthrax.

All this time Burdon-Sanderson still retained his hold upon that wide field of human experience which is offered to every practising physician. He was Assistant Physician to the Brompton Hospital for Consumption from 1859 to 1867, and full physician to the same hospital from 1867 to 1871. At the Middlesex Hospital he was Assistant Physician from 1863 to 1870, and he had, in addition, a certain amount of private practice, although his time for this was necessarily much encroached upon by his scientific occupations. In 1870, however, he definitely gave up clinical practice and all his hospital appointments in order to devote more energy and time to scientific pathology and physiology. The summer of this year is the second great landmark in his career, for, in this, the forty-second year of his life, he succeeded Michael Foster as Professor of Practical Physiology and Histology at University College, London, and, when Professor Sharpey retired in April, 1874, he was appointed Jodrell Professor of Human Physiology, the two chairs being for this purpose merged into one. In order to strengthen the teaching staff, Schäfer, who for several years had been the actual teacher of practical histology, was made Assistant Professor, and entrusted with the histological part of the work, to which was subsequently added elementary physiology.

Burdon-Sanderson held the Jodrell Professorship until 1883, when he went to Oxford as the first Waynflete Professor of Physiology, under a scheme which had been recommended by the University Commission.

The twelve years of his professorial life in London, from 1870 to 1883, were of great fertility from many points of view. As already indicated, his long-continued investigations in subjects connected with public health, led him to forecast that protective treatment, by means of modified sera, which has now become of such great importance; but the duties of a purely physiological chair naturally concentrated his attention more and more upon the phenomena exhibited by normal tissues. He regarded these phenomena as being capable of exact presentation, only in so far as they could be described in the terminology of physics and chemistry; for this purpose it was necessary to utilise the precise methods employed in these sciences, and he spent much thought as to the best way of modifying chemical and physical procedures so as to adapt them for the special purpose of physiological investigation. Even in connection with his previous clinical work, this point of view had come to the front, as is evidenced by his careful investigation of the pulse, for which he made certain modifications in Marey's sphygmograph, and obtained an extraordinary number of sphygmographic records taken in health and in disease. It is also shown by the exact methods he used for determining the amplitude of the respiratory movements, among these being that of a recording stethometer for measuring the movements of the chest wall, the instrument being designed by himself for this purpose.

Probably his earliest strictly physiological inquiry of an experimental kind was that carried out for the Royal Medico-Chirurgical Society in 1862 and 1863 as to the best methods for the resuscitation of the apparently drowned; these investigations led to the adoption in 1864 of the Sylvester method by the Royal Humane Society. Having started on this purely physiological investigation, he was led on to others, and worked upon the respiratory and circulatory mechanisms of mammals for several years prior to his being appointed to the Physiological Professorship at University College.

In 1867 he was elected a Fellow of the Royal Society, mainly for the importance of his researches into subjects connected with public health, but the value of his physiological investigations was also recognised, for in this year he was selected by the Council of the Society to give the Croonian Lecture, the subject being "The influence exercised by the Movements of Respiration on the Circulation of the Blood." In 1872, in conjunction with Foster, Lauder Brunton and Klein, he published the 'Handbook of the Physiological Laboratory,' the special parts for which he was responsible being those devoted to experimental investigations on the circulation and on respiration.

His conception of physiology, as one prominent aspect of general biology, led him gradually into more recondite paths, and he began to devote his energies to the investigation of certain fundamental or elementary aspects of living processes. The nature of these aspects is set forth in the remarkable address which he delivered in 1893 at Nottingham, as President of the British Association. The rule of action of all such processes is there conceived to be "in each instance the interest of the organism as a whole of which it forms part, and the exciting cause some influence outside of the excited structure, technically called a stimulus."

In 1872 he apppears to have commenced a series of experiments into the conditions which determine that universally distributed phase of living tissues termed excitability, and, with his characteristic craving for exact knowledge, he associated with this series the experimental determination of the precise history of an active process from the point of view of its time relations. This involved the accurate measurement of the time occupied by the development, maintenance and subsidence of that excitatory change or state which a living tissue displays in response to an effectual stimulus. The time relations of such excitatory responses are undoubtedly largely dependent upon the character of the active change, and, when this is an alteration in mechanical form or a development of heat, all methods of recording are more or less vitiated by the circumstance that the instrument employed for recording the change has its own personal time; moreover, even as regards the actual tissue, all observed changes of this character are gross ones, so compounded with incidental effects that it is difficult or impossible to discriminate the finer details of the fundamental active alteration.

One type of physico-chemical change, however, appeared to him to approach more closely than others to that which is assumed to take place in the actively responding excitable tissue; this type is that alteration of electrical state which du Bois-Reymond had shown to be recognisable in nerve and which had long been known to occur in muscles and notably in the electrical organs of fishes. Acting on this view, Burdon-Sanderson commenced his well-known researches into the characters of the electromotive phenomena displayed by excitable tissues, and the investigations thus started were destined to occupy him for the remainder of his life, that is for a period of thirty-five years. They form Burdon-Sanderson's most noteworthy contribution to physiological knowledge and illustrate in a striking way the pertinacity with which he investigated a difficult physiological problem and the necessity which he felt for utilising in such investigation recording instruments of the utmost precision. Examples of the latter characteristic are the time and labour which he devoted to devising suitable modifications of the repeating rheotome for use with the galvanometer, and to perfecting the employment for physiological purposes of Lippman's capillary electrometer.

For some time the attention of Darwin had been directed to the phenomena of insectivorous plants, especially his "beloved Drosera: a wonderful plant or, rather, a most sagacious animal." Darwin's work on the digestive powers of Drosera brought him into communication with Burdon-Sanderson, and a letter written in July, 1873, shows that Darwin was struck with the notion that the excitable cells of the plant might exhibit phenomena in all essentials similar to those present in excitable animal tissues. Drosera led to Dionæa, which is characterised by the rapid character of the movement of its leaf lobes when certain hairs upon their inner surface are touched. The exquisite sensibility of the hairs to mechanical displacement and the extraordinarily rapid character of the mechanical alteration in the leaf attracted the attention of Burdon-Sanderson, who saw in this plant an organ admirably fitted for the investigation of fundamental phenomena.

He therefore, about 1875, commenced an investigation as to the changes which were associated with this active process and the conditions which influenced their production and character. In the course of this inquiry he discovered that there was a pronounced electromotive change in the leaf every time it passed into the active state. He then devised an ingenious method for keeping the leaves forcibly open by fixation in plaster of Paris, and demonstrated that although no mechanical movement could now take place when an excitable hair was touched, yet each local stimulus of this kind still evoked an excitatory response, since the same active electromotive change was observed. This active "electrical response" was of considerable magnitude, although of comparatively short duration, and its peculiar interest lay in the circumstance that it was successively developed in all parts of the leaf lobes, indicating the propagation of some active cellular change from the seat of the stimulus to more remote regions.

In 1877 an account of this discovery, together with the experimental determination of the time relations of this electrical response, formed the subject of the Croonian Lecture of that year, the title of the lecture being "The Mechanical Effects and the Electrical Disturbance Consequent on Excitation of the Leaf of *Dionæa muscipula.*" Five years later, in 1882, a more extended and detailed account of the phenomena was published in the 'Philosophical Transactions,' entitled "The Electromotive Properties of the Leaf of Dionæa in the Excited and Unexcited States." From the careful analysis which he had made of his experimental observations, he concluded that the excitatory disturbance, "by the mode of its origin, the suddenness of its incidence and the rapidity of its propagation, is distinguished from every other phenomenon except the corresponding process in the excitable tissues of animals." "In the one case as in the other," he continues, "we must regard the electrical change as a visible sign of an unknown molecular process." The actual mechanical displacement he agreed with Pfeffer in considering as probably related to "the diminution of the turgor or water-charge of the protoplasm of the excitable cells." The experimental evidence which he now brought forward appeared to indicate two possible sources of electromotive change; there was shown to be an initial or primary change, sudden in its development, brief in its duration, and always of the same general type; this he regarded as the electrolytic indication of the peculiar molecular alteration in the plant cells which constitutes the excitatory state; it was succeeded by a second change of longer duration and often of different sign, which was attended by a prolonged residuum or after effect; this he regarded as associated with diminished turgor and consequent displacement of water. It was only by accurate methods of recording that he was enabled to discriminate between the primary and secondary effects and to show that as regards the primary or initial change the active excitatory state is fundamentally the same, whether it occurs in these vegetable cells or in excitable animal tissues.

A third paper upon the electromotive properties of the leaf of Dionæa appeared in the 'Philosophical Transactions' of 1888. This paper included a number of experimental observations as to the behaviour of what he termed "modified leaves"; the observations were made in Oxford, and involved the use of a special double rheotome; the capillary electrometer was also employed, and photographic records of the excitatory electrical changes are given in the paper.

The observations show several interesting facts, but the chief points brought out refer to the so-called "modification," which is produced in a leaf when it is subjected to the flow of even a weak voltaic current. The modification reveals itself as a permanent alteration in the amount, and even the sign of the electrical state of the inactive leaf-surface. It is localised to the part which the modifying current has traversed, and is associated with a remarkable diminution in the high electrical resistance of the tissue, this diminution being strictly confined to the modified region. Finally, the records give demonstrative proof that, when any such modified area is thrown into the active state, either by direct stimulation, or by the arrival of a propagated excitatory wave aroused elsewhere, then the electrical response in this area, whilst it has the same time relations, may be entirely changed as regards its sign. This extraordinary reversal is related to the sign of the inactive tissue, and, when through a modifying agency this is altered, then the sign of the active change may be similarly reversed.

From the constancy of this relationship, Burdon-Sanderson inferred "that the constantly operative electromotive forces, which find their expression in the persistent difference of potential between the opposite (leaf) surfaces, and those more transitory ones, which are called into existence by stimulation, have the same seat, the opposition between them being in accordance with the general principle that, whereas the property which renders a structure capable of undergoing the excitatory change is expressed by *relative positivity*, the condition of discharge is expressed by *relative negativity*."

Between the first and second papers on Dionæa he had, whilst at University College, London, pursued the same method of inquiry in the cardiac muscular tissue of the frog. His investigations upon this subject are set forth in a paper published in 1880 in the 'Journal of Physiology.' These researches have been taken as a model for most subsequent work of this character, whatever the tissue which has been utilised for such electro-physiological investigations. The paper is entitled "The Time-relations of the Excitatory Process in the Ventricle of the Heart of the Frog," and was followed three years later by one "On the Electrical Phenomena of the Excitatory Process in the Heart of the Frog and of the Tortoise, as Investigated Photographically." In both these investigations he had the assistance of Mr. F. J. M. Page. The whole research has been long regarded as classical, through the exactitude of the methods of observation, the rigorous determination of the influence of various surroundings or accessory factors, and the lucid interpretation of the phenomena. It conclusively demonstrated that the diphasic effect (or change of electrical sign) which is observed when two contacts are placed on the uninjured surface of the frog's heart, is the algebraic sum of two monophasic electromotive changes each of similar sign but developed successively one under each of these two contacts. Hence it is not an indication of the occurrence of opposite types of tissue change, but is simply the expression of a similar type of monophasic change occurring at different times in different localities. Finally, he demonstrated that the reason why the change begins under one contact before it occurs under another one, is solely because the excitatory state, like the muscular contraction, is propagated in the differentiated cells which compose the cardiac tissue. For these researches he was, in 1883, awarded a Royal Medal.

After his appointment to the Oxford Chair in 1882, Burdon-Sanderson had to brave the turmoil of an opposition in which an antivivisectionist outcry was utilised. This opposition was directed successively against a proposed expenditure of £1,500 for apparatus in 1882, and of £10,000 in 1883 for erecting an appropriate physiological laboratory. In 1884 it culminated in an attempt to throw out the decree authorising a maintenance grant of £500 a year for the incidental expenses of the newly erected Physiological Department. The details of the scenes which were associated with the debates upon the subject in Convocation are now almost forgotten, and it is unprofitable to revive them. It will be sufficient in this memoir to say that, in spite of the virulent character of the feeling displayed by his opponents, it was generally felt that Burdon-Sanderson came through the storm with, if anything, an added dignity, both opponents and supporters being impressed by his self-control under circumstances which might have justly provoked his resentment. One who knew him well at this time, Sir William Church, wrote that he would "never forget his striking face and figure as he stood quietly leaning against the wall of the Sheldonian Theatre during the momentous debate in Convocation on February 5, 1884."

The establishment of Burdon-Sanderson in Oxford, with the erection of the Physiological Laboratory, and thus the foundation of that scientific study which used to be called the Institutes of Medicine, may be regarded as the third landmark in his career. It is true that it was to Sir Henry Acland that Oxford owes the idea of resuming its part in medical education, but with Sanderson's arrival the creation of the Oxford Medical School passed from a fruitful idea into reality, and it is now generally recognised that he achieved the end for which he left London by establishing on an enduring, because a scientific, foundation, the Oxford Medical School. He thus enjoyed the rare felicity of living to see the purpose which he had so much at heart, in all essentials, attained. An excellent Department of Human Anatomy was erected during his Physiological Professorship, and when, in 1895, he resigned the Physiological Chair, on his being nominated Regius Professor of Medicine, he devoted his energies towards the efficient equipment of pathology. The scientific progress of this subject had always been one of his great aims, and his satisfaction was unbounded when, through the stimulus of a generous donor, the University acknowledged its importance and erected an adequate laboratory. He gave not only time and thought but material help towards the task of making this new University department an efficient one, and left in his will the sum of £2,000 in the hands of trustees "for the support of the Laboratory of the Pathological Department of the University of Oxford and specially to provide for the expenses of research in pathology conducted in the said laboratory or elsewhere."

The most extensive of the researches which he carried out at Oxford were upon muscle. In order to obtain exact records of the electromotive changes during muscular activity, he devoted great attention to the working out of an adequate method. Inspired by his enthusiasm, Burch took in hand the capillary electrometer, greatly improving its construction and developing a method by means of which the graphic records of its well-known displacement could be interpreted with accuracy. It thus became possible to construct, from the actual photographic records, other derived curves which should faithfully display the variations in the potential difference of a tissue even when these variations were of very brief duration.

The whole method was elaborated by Burdon-Sanderson with scrupulous attention to accuracy of detail; he left it the most exact method of its kind employed in this or any other country, and the utility of the technique has now received ample acknowledgment from all those who are engaged in investigations upon the electromotive phenomena displayed by excitable tissues. Others, inspired by his example and encouraged by his help, have applied the method with success for the elucidation of the electromotive changes occurring in nervous and other tissues, including those which are found in the electrical organs of fishes. As regards the last-named structures, Burdon-Sanderson himself visited Arcachon in 1886, St. Andrew's in 1887 and Plymouth in 1888, in order to investigate the electrical organs of fishes, and published, in conjunction with F. Gotch, two papers upon the electrical organ of the Skate in the 'Journal of Physiology.'

The results of the investigations upon muscle are given in the papers which he published in the 'Journal of Physiology,' and a comprehensive account of the work and of his conceptions as to the excitatory process in this structure, is set forth in the third of his Croonian lectures.

This lecture, delivered in 1899, was entitled "The Relation of Motion in Animals and Plants to the Electrical Phenomena associated with it." It deals first with the technique by means of which he endeavoured to obtain precise information and then sets forth the main results obtained by its employment in Dionæa and in striped muscle. The electrical response is described in various forms of muscular contraction such as twitch, tetanus and reflex activity, also in the peculiar condition produced by veratria. In summarising the indications which the phenomena seemed to him to afford, he stated that "in striated muscle the primary effect of every excitation is a process of oxidation having its seat at the excited part. It may be surmised that this consists of two stages, namely, liberation of previously intramolecular oxygen, and actual oxidation." He further concludes that "the monophasic variation may be taken as the type from which all other forms of response to stimulation may be derived, either by repetition, prolongation or interference."

An interesting point for electro-physiologists is associated with the concluding sentence, which affirms that he believes the "muscle current" to be "the manifestation of processes which have their seat at the surface of contact between electrode and living muscle"; he thus definitely adopted the view which is termed "præ-existent" as opposed to the "alteration" conceptions of Hermann. The work upon reflex muscular activity was continued almost up to the time of his death; a brief note relating to the phenomena of strychnia tetanus in the 'Proceedings of the Physiological Society' of 1902, is, however, the only publication on the subject, although a large mass of information had been obtained.

It is only possible within the limits of this notice to allude quite briefly to the many suggestive articles and addresses which are scattered along the years of his scientific activity. They are chiefly related to the consideration of pathological and physiological subjects; notable ones are the Addresses delivered as Sectional President at the British Association in 1872, 1881, and 1889, the Address on the Cellular Pathology of To-day, delivered to the Thirteenth International Medical Congress at Paris in 1900, and the masterly Address, delivered in 1893 at Nottingham, as President of the British Association. Among his pathological articles, probably the most conspicuous is that on "Inflammation," in Holmes' 'System of Surgery,' which, although written early in the seventies, is still accepted as the classical utterance upon this subject. An extensive article of a physiological character was written in 1900 for the 'Textbook of Physiology,' on "The Mechanical, Thermal and Electrical Properties of Striped Muscle."

Viewing his scientific work as a whole, the most remarkable feature which it presents is the breadth of its scope: sanitary science, practical hygiene, medicine, pathology, bacteriology, botany and physiology have all been enriched by his activity. If one of these branches can be selected as having been more particularly illuminated by his genius, the choice must fall on pathology. In this his influence was unique; he was ahead of all his contemporaries in this country, both in conception and in method, and by leading his successors into the true path—that of experimental investigation—he endowed English pathology with purpose, and gave this subject, as regards this country, its proper scientific status. His influence upon physiology, although profound, was not of such an epoch-making character; in one respect, however, it has been most marked, and that is in connection with the methods now adopted by all physiological teachers. It has been suggested that Burdon-Sanderson was great as an original investigator rather than as a teacher, but this suggestion is far from being borne out by fact. A great teacher is to be judged by the ability and subsequent eminence of his students and by the radical alterations which he has been able to initiate in teaching methods; from both these points of view Burdon-Sanderson achieved greatness. His lectures were extraordinarily suggestive, whilst his personality and grace of expression held his audience. But a more solid tribute to his teaching capacity is the change in teaching which he was the first to effect, and which consisted in the introduction into the students' course of practical work and thus of the experimental method.

As Jodrell Professor in University College, London, he organised classes for the practical study of physiological chemistry and of the phenomena of muscle and nerve. The printed syllabus of the work to be done formed an appendix to the published synopsis of his physiological lectures; it was the first of its kind, and inaugurated a system of practical instruction which now forms part of the routine work in every University existing in this country. For many years the methods employed by him for the teaching of students were, in this respect, in advance of those which existed upon the Continent.

The stress which he laid upon this part of physiological training was the logical outcome of his firm belief in the application of experimental methods to medical science. Quite apart from the obvious advantages which such practical teaching offers through its facilitating the acquisition of certain facts, work of this character by students appeared to him of the utmost value in engendering a mental attitude of primary importance. He believed that it cultivated those powers of observation, experimental inquiry and reflection, in regard to living phenomena, which have subsequently to be brought to bear on the human subject by all those who undertake the responsible duties of clinical practice. Throughout the whole of his life he held that, since medical science remained inexact and empirical unless it rested upon the sure foundation of physiological and pathological knowledge, and since this knowledge could itself be advanced only by experimental methods, the highest interests of society were bound up with the practice of such methods. Whether or not infectious diseases are a product of civilisation, they are certainly fostered by man's social habits and communicated by him to animals, and the responsibility lies with man to utilise to the utmost his mental powers for their removal or delimitation. This responsibility was appreciated in its fullest extent by Burdon-Sanderson, and all his work and teaching was tinged with his endeavour to faithfully discharge the trust which his position and mental powers involved. He had in consequence an earnestness of purpose which was felt by all those who came under his influence. The words in which he set forth, in 1896, the greatness of Ludwig as a teacher, are applicable to himself: men, many of whom were already nearly of his own age, rallied round him, "attracted in the first instance by his discoveries, they were held by the force of his character, and became permanently associated with him in his work as his loyal friends and followers, in the highest sense his scholars." The large number of his students who now hold official appointments in connection with medicine, surgery, hygiene, pathology, and physiology, will acknowledge the truth which the foregoing sentence expresses.

The present writer is one of those who, as a student, came, in 1874, under the sway of his inspiring personality, and on reflecting as to the various factors which gave this sway its extraordinarily potent character, it would appear that the following features may be specially distinguished. His commanding appearance and striking individuality; the intellectual beauty of his face, which is so well displayed in the print copied from a photograph taken by Miss Acland, at Oxford; the peculiar charm of his manner and diction; the sympathetic interest with which he followed the work and thought of others, young or old; above all, that earnestness of purpose to which allusion has been already made. Like Ludwig, he strove "to unite his life-work as an investigator with the highest kind of teaching." This life-work, extending for over fifty years, coincided with what is generally regarded as the most eventful period in science; it is undoubted that as regards biology, physiology, and pathology, the latter half of the nineteenth century has been creative. The propitious moment in these provinces of science arrived about the year 1850, and with the birth of the new movement came the men fitted by their endowments to accelerate its progress. The part which Burdon-Sanderson took in directing the course of this movement as regards pathology and physiology, stamps him as one of those masters who mould the thought of a generation.

Various distinctions, unsought by him but valued as indicating public appreciation of work done for science, were bestowed upon him. He was selected on five different occasions to serve on the Council of the Society, was awarded a Royal Medal, was appointed Croonian Lecturer on three separate occasions, was the recipient of many honorary degrees, was made a member of the chief Foreign Scientific Academies, and in 1899 was created a baronet. His appreciation of these distinctions caused no change in those traits which endeared him to the large circle of his friends, for he always retained that charming simplicity of character and of life which, when associated with great intellectual gifts, increases the affectionate and reverent regard of others.

He was a man of wide sympathies, intellectual and artistic, with an appreciative taste in art and music which was only realised by his more intimate friends.

The breadth of his views and the commanding position which he held in the scientific world augmented the regard in which he was held by the University of Oxford and by the particular society, Magdalen College, with which he was associated for twenty-three years, first as Professorial and later as Honorary Fellow. The peculiar charm of his individuality and character secured the devoted attachment of a wider circle. There are many to whom he was that "true friend to whom you may impart griefs, joys, fears, hopes, suspicions, counsels, and whatsoever lieth upon the heart to oppress it," many to whom the memory of his intimate friendship will remain as one of the abiding joys of life.

During his tenure of the Regius Professorship of Medicine at Oxford, the condition of his health was several times such as to cause his friends much uneasiness, and particularly when, at the close of the year 1903, he felt compelled to resign the Chair. It was a source of unbounded satisfaction to him to realise that, by the appointment of Dr. Osler as his successor, the continued development of medical study in Oxford was assured, and that the task set before him when he left London in 1882 was practically accomplished. For him, as for many others who have spent their lives in the service of science, the words of Bacon form an appropriate epitaph—"But, believe it, the sweetest Canticle is *Nunc dimittis*, when a man hath obtained worthy ends and expectations." F. G.

(The photograph which is reproduced in the frontispiece to this account was taken by Miss Acland, at Oxford, in the year 1894, who kindly lent the negative for the use of the collotypers.)

HENRY BENEDICT MEDLICOTT, 1829—1905.

HALF a century ago, our knowledge of the Geology of India was very small Some traverses of the Himalayas had been made, and notes had been published of portions of the Peninsula of India; fossils had been recorded and described from Spiti, the Salt Range, Sind, Cutch, Trichinopoly, and a few other localities; coal had been found and to some extent worked, but very little was really known of the different formations or of their relations to each other. A comparison of Dr. Carter's 'Summary of the Geology of India between the Ganges, the Indus, and Cape Comorin,' published in 1853, or of Greenough's geological map (1854), with the Manual of the Geology of India issued in 1879, and with the map accompanying it, will show the great difference between the knowledge of Indian Geology at the two periods.

Amongst the men who originated the Geological Survey of India and by whom the great improvement in our acquaintance with the geology of the country was secured, a leading position was taken by Henry Medlicott. More of the numerous errors which had become almost stereotyped amongst Indian geologists were corrected by him than by any other surveyor. To take one example only. In the Peninsula of India there are, in the classification adopted in the Manual, the following groups of formations below the great Deccan basaltic series (Upper Cretaceous and Lower Tertiary):—(1) Metamorphic or Gneissic; (2) Transition; (3) Vindhyan; (4) Gondwána; the (5) Marine Jurassic and (6) Cretaceous beds being confined with one unimportant exception to coastal fringes. The Vindhyan and all below it are now believed to be pre-Cambrian; the Gondwána ranges from Upper Palæozoic to Lower Cretaceous. Now in the 1854 'Summary of the Geology,' all the Vindhyan and Gondwána, various portions of the Transition Rocks and most of the known limestones from Metamorphic to Cretaceous were assigned to the "Oolitic Series." In his first season's work with his brother, J. G. Medlicott, Henry Medlicott distinguished the pre-Cambrian Vindhyan from the Gondwána, thus at once clearing the way for further progress. In his first published Memoir he arranged in sequence the Vindhyan, Sub-Kymore or Lower Vindhyan and Bijawar or Transition of Bundelkhand, and distinguished all from the Gneissic Series. In his next Memoir he classified the rocks of the Himalayas, both the Siwaliks and Nahuns, resting on the Nummulitic Subáthu group, and the ancient hill rocks, Krol and Blaini, associated with the older formations of the mountains.

Henry Benedict Medlicott was born in Loughrea, County Galway, Ireland, on August 3, 1829. He was the second of three sons of the Rev. Samuel

Medlicott, Rector of Loughrea, and of Charlotte, daughter of Colonel H. B. Dolphin, C.B.; his elder brother, Joseph G. Medlicott, was also for several years on the Staff of the Geological Survey of India.

Henry Medlicott was educated in France, Guernsey, and Dublin, where he graduated in Trinity College and took the degree of B.A. in 1850, with diploma and honours in the School of Civil Engineering. He took the M.A. degree in 1870. He was thus from his early education an excellent French Scholar, and his acquaintance with the classical works of the great French geologists may be often traced in his writings.

In 1851 he joined the Geological Survey in Ireland, and in 1853 he was transferred to the English Survey where, for a time, he worked with Mr. Aveline in Wiltshire. He was next engaged by Dr. Oldham and joined the Indian Geological Survey, then occupied in the Rajmahal Hills at or near Bhagalpur on March 24, 1854. His brother, J. G. Medlicott, had joined the survey a year or two earlier. Before, however, Henry Medlicott had commenced field work, he was appointed, in August, 1854, by the Court of Directors of the East India Company, Professor of Geology in Rurki College, and he remained in this post till October, 1862, when on some additions being made to the staff of the Geological Survey, he rejoined it as Deputy Superintendent for Bengal.

During his tenure of the Rurki Professorship, however, he did most important work in field geology. By an arrangement with Dr. Oldham he was allowed to occupy the field season in surveying, and thus he was enabled to examine parts of the Nerbudda Valley and Bundelkhand in 1854—55 and in 1856—57, whilst in the other years he was engaged in working out the geology of the Lower Himalayas between the Ganges and the Ravi (Hardwar to Dalhousie), and of the Siwalik Beds and their associates at the base of the mountains. In 1857 he served as a Volunteer with the garrison of Rurki against the mutineers of the Bengal Army, and on the close of the Campaign was awarded the Indian Mutiny Medal for Special Service. An instance of the service rendered may be added here.

Rurki during the outburst of the Sepoy mutiny was isolated, like most of the small garrisons in the Upper Provinces, where the natives of the villages were associated with the rebels. No European could possibly traverse the country in safety. News was received at Rurki one day of a Christian family who were held by the villagers as prisoners at a few miles distance, and who were in imminent danger of being killed. It was arranged that Henry Medlicott and one European companion should go out with an escort of Sowars to endeavour to rescue these unfortunate people, but on the day before the attempt the Sowars showed signs of mutiny and were disarmed. Medlicott and his companion, whose name appears now impossible to trace, left in the morning, and by sheer pluck, at the risk of their lives, succeeded in bringing back the imprisoned people in safety, the villagers apparently being so astonished at two Europeans coming among them that they surrendered their prisoners. Only the outline of the story can be now recovered; it was told to the present writer in 1866 by Colonel Baird Smith, who commanded the Rurki Garrison in 1857.

On October 27, also in 1857, Mr. Medlicott was married at Landour to Louisa, daughter of the Rev. D. H. Mannsell. He has had three sons and three daughters; only one son and one daughter, together with his wife, survive him. The surviving son is a Civil Engineer in the Pubic Works Department of the Indian Government; the two who have been lost were one in the Civil Service of India and one in the Royal Engineers.

After rejoining the staff of the Geological Survey in 1862, Mr. Medlicott was engaged for many years, first in one part of the country then in another, generally by himself, though occasionally in charge of a party, in enquiring into economical problems or in investigating geological questions, and not unfrequently in clearing up difficulties that had proved too serious for his predecessors. In 1862—63 he was in South Rewah, in 1863—64 in Behar, in 1864—65 he traversed Assam, from the Khasi Hills to the farthest eastern extremity of the province, in 1865—66 he was engaged in Central India and Rajputana. In other years he examined various tracts of Northern India from the Jamu Hills of Kashmir, the Satpura ranges of the Central Provinces and Chattisgarh to the Garo Hills in Assam. Twice he officiated as Superintendent of the Survey during Dr. Oldham's absence on leave, once in 1870 for a short period, and in 1873—74 for 17 months.

On April 1, 1876, Mr. Medlicott succeeded Dr. Oldham as head of the Department, and from that time the duties of his office kept him mainly in Calcutta. The title of Superintendent was altered to that of Director in 1885, but the change was confined to the title.

Dr. Oldham had been in weak health for some time before he retired, and he naturally left many arrears for his successor to carry out. Foremost amongst these was the preparation of a general account of Indian geology. This had long been required; a mass of scattered papers and reports, not a few of which were in MS., represented about a quarter of a century's progress with the systematic examination of Indian geology, and before those who had helped from the beginning had left India for good, it was essential that they should prepare for their successors a record of work in which they had taken part. Moreover, until a general description of the country, as a whole, was compiled, it was impossible for any one either in India or in Europe, without an enormous amount of labour, to gain a correct idea of its geological features. Henry Medlicott at once set to work at this general account, and dividing the chapters between Mr. Blanford and himself, brought it to a conclusion in 1879 and published the 'Manual of the Geology of India,' with a map, in that year. It is unnecessary to point out the great importance of this book, nor the assistance it has afforded, not merely to Indian geologists but to those throughout the world.

This, however, is only one of the subjects to which the new Superintendent of the Survey devoted his attention. Of the effect of his superintendence on the work of the staff a fair example may be furnished by the survey publications.

From 1877 the records which, as describing recent work, are the best illustrations of survey progress, doubled in size, and they certainly did not fall off in the importance of the contributions printed, either economical or scientific. Besides these Mr. Medlicott edited, during his tenure of office, about 10 volumes of the Memoirs and numerous parts of the Palæontologia Indica, comprising Dr. Feistmantel's descriptions of Gondwána plant fossils, most of Mr. Lydekker's accounts of the Siwalik and other vertebrata, and the largest portion of Dr. Waagen's great work on the Salt Range fossils, and of the descriptions of Tertiary Corals and Echinoderms from Western India by Dr. Duncan and Mr. Percy Sladen. There was the same thorough progress in other directions: for instance, in the arrangement of the Museum and of the Survey Office.

His work absorbed him entirely; living almost the life of a hermit, and confining himself to his office, he devoted all his time to the Survey. He was a man of a retiring disposition originally, not in the least from want of energy or courage, but because the social pursuits of men in general did not attract him; he had, in fact, something of the ascetic temperament. During his life at the Museum, he not merely lived alone on the poorest of fare, but he scarcely quitted the building. In the evening he took exercise on the roof. He had retained some early habits, such as walking bare foot, and long after he left Calcutta, a path along the flat Museum roof, worn by his naked feet, could still be traced. As a young man he had been strong and active, and amongst other traits had acquired the power, not often possessed by Europeans, of sitting like a native of the East on his heels. His method of living he rarely cared to explain, though as a fact, whilst his own living expenses were reduced to a minimum, far below the usual minimum of Anglo-Indians, he was providing for several relatives and not merely for his own family. The death of his brother, too, had added to a considerable extent to his responsibilities.

After the completion of his 55th year, in 1884, Mr. Medlicott was retained by the Government of India in his office until 1887. He retired on April 27 in that year, having served almost continuously for rather more than 33 years. He was only on leave for six months in 1865, less than six months in 1871, and again six months in 1884, or 18 months during the whole period of service.

Since his retirement he lived very quietly at Clifton, near Bristol, and had taken but little part in scientific discussion, though always keenly interested in the progress of the Indian Survey. His time, as long as he remained in good health, was mainly devoted to philosophical problems, to which he had always been devotedly attached. He published two short pamphlets on "Agnosticism and Faith," in 1888, and on "The Evolution of Mind in Man," in 1892, and was engaged on a larger work which has not been finished. A few years since, a strain caused by bicycling, when, with characteristic energy and determination, he had resolved that he would not be beaten by a particular hill, led to serious heart symptoms, and, although for a time a partial recovery was made, a relapse early in 1904 reduced him greatly.

The end came quietly on April 6, 1905, when he was seated in his study.

Henry Medlicott joined the Geological Society of London in 1856, and received the Wollaston Medal in 1888, on his retirement from the Indian Service. He was elected a Fellow of the Royal Society in 1877. He was a Fellow of Calcutta University, and from 1879 to 1881 he was President of the Asiatic Society of Bengal. He was also an Honorary Fellow of several Foreign Scientific Societies, but he never made use of his titles, which he regarded as simply "official." It may be remarked that he carefully omits the letters F.R.S. after his name in all his survey publications, and even in the "Manual" he struck out the initials after his own name, whilst leaving them after his colleague's. Throughout his career, in fact, he appears almost unnecessarily to have disregarded the honours to which he was entitled for brilliant original work as well as good official service.

His policy as head of the Survey was to assist his colleagues in every way to publish the account of their observations in their own words. Occasionally the result was that their views drew upon the authors replies from other officers who took different views, or from geologists who had another opinion on the subject. This was especially the case in palæontological questions, on which he never pretended to decide difficult points. The policy adopted is perhaps not quite in accordance with official usage, but there can be no doubt that the result of Mr. Medlicott's principle was to bring out the facts in a discussion, and not infrequently in a difficulty of high scientific and economical importance, as in the various disputes over the Gondwána flora. And not only did he obtain the help of the staff of the Survey in forwarding the progress of Indian geology, but he succeeded in securing the valuable assistance of the late General McMahon and others, who contributed observations on subjects of high interest in the geological history of the Indian Empire. That he contributed no lengthy memoirs of his own, was simply due to the fact that he regarded with horror any attempts to gain credit by others' work. Short notes and an annual report were for many years his principal contributions to the Survey publications. He was not a fluent writer, though he could write strongly and earnestly, never so strong as when he was dealing with attempts at injustice or plausible but misleading statements. He was absolutely fearless, and cared but little of whose pet theory he was disposing, if the theory was in his opinion worthless.

It is difficult to appreciate Henry Medlicott's work in India without some acquaintance with the knowledge of the geology available when he entered the service. So great a change has taken place in the half century that has elapsed since 1854, that it is scarcely possible to reconstruct the conditions under which geological surveying was carried out 50 years ago. There were no railroads and very few roads. Travelling was difficult and very slow, the early surveyors had often to make their own maps in the wilder parts of the country. Some idea of the Geology known in the days before the Survey may be gained from Dr. H. J. Carter's 'Summary,' which appeared in the same year that Mr. Medlicott landed in India. In this remarkable work limestones from every formation in India, from metamorphic to the Bagh and Intertrappean beds, are included in the "Oolitic Series." An equally remarkable confusion is shown upon Greenough's Geological Map of India.

Nothing had been done to clear up the nebulous condition of Indian Geology before 1854. Henry Medlicott's first season in India was signalized by the earliest and most important step in the classification of the Peninsular rocks, when he and his brother separated the Vindhyans north of the Nerbudda Valley from the Gondwánas to the south. The name Vindhyan was given by Dr. Oldham, who, however, when announcing the discovery,* stated that the separation had been made by the Medlicotts before he visited the country. In the first memoir he wrote, in Vol. 2 of the Memoirs, Henry Medlicott reduced the whole mass of Vindhyan, Infra-Vindhyan, and Bijawar rocks that extend throughout Bundelkhand to a sequence which has received no great subsequent alteration, the principal change, perhaps, being in the substitution of the term Lower Vindhyans for Sub-Khymores. This masterly paper, though published in a most imperfect condition, ill-arranged, and not very clearly written, was not only the beginning of our accurate knowledge of the Vindhyan and Infra-Vindhyan rocks, but laid a firm foundation on which much has since been built. From his earliest work to his latest, these wonderful azoic rocks of India were Mr. Medlicott's especial favourites, perhaps because all the knowledge of them was derived from purely physical observations, and not interfered with by organic remains, which in the later Gondwánas have not always proved an accurate indication of the age of the beds or their relations to each other. Throughout his writings in India, and throughout the Annual Reports issued during his Directorship, point after point is brought up tending to the correlation of these formations in various parts of India, and showing the relations between Karnul and Cuddapah, Kaladghi, Gwalior, and many other similar rocks on the one hand, and the Vindhyan and Bijawar on the other.

His next great work was the arranging of the rocks of the Lower Himalayas in the Simla and neighbouring area and of the Siwalik rocks and their associates at the base of the mountains, and the sketching of the geological history of the Himalaya range on a definite geological basis. The first has been one of the most difficult questions in Indian geology, and although the work was commenced in 1855, it cannot be regarded even now as nearly solved. The Tertiary Himalayan Beds have, however, been regarded as fairly classified since Mr. Medlicott's Memoir was published in 1864. The important observations made in this Memoir are essentially physical. The demonstration that Himalayan elevation is shown by the relations of the Lower Nummulitics and the older hill rocks not to have begun before Tertiary times, and the beautiful illustrations proving the permanence of the great river valleys by their coincidence with the maximum of conglomerates in the Siwaliks, are amongst the observations that connect our first clear ideas of Himalayan elevation with Henry Medlicott's work.

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* 'Journ. Asiat. Soc. Bengal,' 1856, vol. 25, p. 250., and more clearly in 'Mem. Geol. Surv. Ind.,' vol. 2, p. 304.

It has been already noticed that not unfrequently Henry Medlicott was occupied in clearing up difficulties that had been too great for his predecessors in the Survey. An illustration may be taken from the geology of the Khasi Hills. A comparison of his observations as recorded in the seventh volume of the Memoirs should be made with the earlier account of the same area by Dr. Oldham in the first volume. In the first account, amongst other differences, the bedded Sylhet traps (Mesozoic) are not distinguished from the ancient greenstone rocks of the inner hills, and the occurrence of fossiliferous Cretaceous rocks was not recognized; their fossils, then supposed to have been Nummulitic, having been lost. The alterations made in the geology by Henry Medlicott may be seen by comparing the map and section, Plate 8, in Vol. 1, with the map and section at p. 154 in Vol. 7. The earlier observations, it is true, were made in the monsoon, when of course the ground was not as well exposed. Again, the sketch map of the Satpura or Nerbudda coal area as corrected by him in Vol. 10 of the Memoirs may be compared with the earlier map published in Vol. 2. It is quite true that the principal discrepancy in this case is easily explained. J. G. Medlicott, who not merely geologically examined the country but who also to a considerable extent surveyed the map, had practically completed the western portion containing the typical Mahadevas without separating them from the underlying Damudas. In the early part of 1856, Dr. T. Oldham, the head of the Survey, went over the field, and almost at the end of the season discovered that the Mahadeva was a separate series. He desired that this separation should be recorded on the map. The result is, what may be supposed under the circumstances, complete chaos, from which Henry Medlicott first rescued the mapping of the Gondwánas. It is scarcely necessary to say that portions of his Report were omitted by the then Superintendent of the Survey from the printed copy, as he has mentioned in the Annual Report for 1880.*

In fact, throughout his life Medlicott had been the champion of whatever he believed to be founded on scientific truth. Almost his only important geological paper, outside of the Survey Publications, was that on "The Alps and the Himalayas, a Geological Comparison," issued in the 'Quarterly Journal of the Geological Society' for 1868, Vol. 24. This Paper narrowly escaped rejection; it was postponed for a time, but was finally published. Nor was this hesitation wonderful, for the paper attacks, and in no doubtful way, the conclusions of all the great Alpine geologists, v. Hauer, Gumbel, Studer, Desor, and others, and there is no question that in the main Medlicott was right. Some of the views expressed by him required and have since received revision, but as an original description of mountain-building from a uniformitarian as opposed to a catastrophic point of view, it deserves far more attention than it has received.

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* 'Rec. Geol. Surv. Ind.,' 1881, p. 1.

There can be no question of his strong uniformitarianism. One of his objections to some of his colleagues' work was against the use of faults to explain abrupt boundaries. It was, however, characteristic of his love of truth that when Mr. R. Oldham found that the typical Nahun-Siwalik section, to which Henry Medlicott had so long referred as an example of deposition against a pre-existing cliff, might after all be a simple fault, he revisited the section and in the simplest manner admitted that he had misunderstood it. Only those who remember the whole controversy about faults can have an idea of how great a blow to his theories this must have been, and from some experience of Directors of Surveys, there are few of whom it could be said, that an error of so much importance was so frankly acknowledged. On the larger question, whether, for instance, the great lines terminating the Gondwána basins of Bengal, Orissa, and the Central Provinces are simple faults, or whether they represent the boundaries of the old river valleys in which the Gondwánas were formed, and to which they were limited, these boundaries having subsequently been slightly crushed and dislocated, but not greatly faulted, no complete answer has yet been given. In some cases at least, for instance, in the Mopani field of the Satpuras, strong evidence was brought forward by Henry Medlicott in favour of his view of original limit, and even in the case of Raniganj, where unquestionably considerable faulting exists, the absence of outliers of the coal-bearing rocks south of the field is a difficulty in supposing that a simple great upthrow alone terminates the Gondwána area. Between the two authors of the Manual of Indian Geology there was a difference of opinion on this point, and it cannot be said that the question is completely settled.

The above may serve to recall a few of the services of Henry Medlicott to the Geological Survey and to India, and some of the discoveries which he made in science. It cannot do more than suggest the amount of labour that he devoted to his work. His memory should remain as a striking example of the thoroughly honest and capable geologist and as a worthy head of a scientific branch of the Indian Government.

W. T. B.*

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* [A melancholy interest attaches to this Notice by Dr. W. T. Blanford. It was written by him at the request of the Council, and the proofs were duly sent to him, but they arrived when he was already too ill to look at them, and he died before they could be printed off and published. His genial sketch of his old friend and colleague was thus the last task he accomplished in his own life.—Sec. R.S.]

WILLIAM THOMAS BLANFORD, 1832—1905.

William Thomas Blanford, though much of his working life was spent out of his native land, began and ended his days in London. He was born on October 7, 1832, at 27, Bouverie Street, in a house which, with the adjoining manufactory, had been built by his father, William Blanford, the site being now occupied by part of the offices of the *Daily News*.

From private schools at Brighton he went, at the age of 14, to one in Paris, and there had a serious illness, probably diphtheria, just at the time of the revolution of 1848. On leaving school he spent two years at Civita Vecchia in a mercantile house, and on his return to England, in 1851, joined his father's business. But his younger brother, Henry F. Blanford, afterwards the well-known meteorologist, and a Fellow of this Society, had already become a student at the Royal School of Mines, and the elder one before long yielded to the same attraction, and commenced his studies there in 1852. During the vacations they worked at field geology with Ramsay (afterwards Sir Andrew) in Wales, and mining, with Smyth (afterwards Sir Warington), in Cornwall. Each passed out head of the list for his year, obtaining the Duke of Cornwall and the Council Scholarships.

W. T. Blanford, on leaving the school in 1854, continued his studies at Freiberg in Saxony, but next year both brothers were offered and accepted posts on the staff of the Geological Survey of India, then in charge of Dr. Thomas Oldham, arriving in Calcutta at the end of September.

Early in 1856 they, with an older officer, were sent to examine a coalfield near Talchir, in the province of Orissa, south-west of Calcutta. The results were important, though the coal was of little value, for they were able to distinguish the measures containing it—afterwards called the Damuda series —from an upper group which they named the Mahadeva and a lower one, the Talchir, all forming part of the Gondwána system now familiar to geologists. They also found that the Talchir group contained boulders sometimes 4 or 5 yards in diameter, embedded in a fine silt with smaller stones, facetted and striated, which, they suggested, must have been transported by ice. For some time the idea of a glacial epoch in late Carboniferous or early Permian times was not received with favour, but it has since received ample confirmation.*

Afterwards Blanford visited Darjiling, and helped in surveying the Rajmahal Hills, having a narrow escape from a band of mutineers on his return to Calcutta, where he joined the Volunteer Cavalry Guards. From the later part of 1857 to 1860 he was engaged in resurveying the Raniganj

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* The subject was fully discussed in W. T. Blanford's address to the Geological Section of the British Association at Montreal in 1884.

Coalfield, and in visiting the Trichinopoly district and the Nilgiri Hills, but in the cold season of the latter year he was sent to commence a geological survey in Burma, where he visited Mandelay, not then in British territory, and made zoological collections which added much to the knowledge of the Burmese fauna.

Promoted after a furlough in England in 1862 to be Deputy Superintendent, Blanford had charge of the survey of the Bombay Presidency, where he was engaged for the next four years, also visiting Sind and Cutch. The position of the Deccan traps in regard to the underlying Cretaceous and the overlying Nummulitic series was determined, as is described in the sixth volume of the 'Memoirs of the Geological Survey of India.'

In September, 1867, Blanford was attached to the Abyssinian Expedition to study the geology and zoology of the country. Landing at Zeila shortly before Christmas, he accompanied the army to Magdala, afterwards remaining for some time in the Bogos province. On his return to India in the autumn of 1868 he was released from field duties in order to work out his collections, and then spent six months in England to complete his 'Observations on the Geology and Zoology of Abyssinia.' After returning to India in the late autumn of 1869, he visited Sikkim next year with Captain Elwes, getting as far as the Tibetan frontier, and was then engaged in the Godavari Valley and the border of the Madras Presidency, chiefly in the coalfields, returning to Calcutta when the hot weather began. By this time he had traversed the whole peninsula on foot or on horseback, with the exception of some 20 or 30 miles, from the Arabian Sea near Surat to the Bay of Bengal at Coconada.

In the autumn of 1871 he was ordered to join the Persian Boundary Commission, and after a preliminary voyage to Basra and back started from Gwadar, about 300 miles west of Karachi, marched to Shiraz with Major St. John's party, and then travelled alone through Ispahan to Teran to join the other one under Sir Richard Pollock. After a short visit to the Elburz Mountains, he returned to England from Enzeli on the Caspian, by Astrakan, Moscow, St. Petersburg and Berlin to England, arriving at home in September, 1872. Here he was obliged to remain on a two years' leave, for his health had suffered from the hardships of his work in Persia, but he was fully occupied in preparing the volume on the 'Zoology and Geology of Persia' for the Reports of the Boundary Commission.

After his return to India at the end of 1874, he was engaged during the next three years on the geology of Sind, except for a three months' exploration of the desert between the Indus and Jodhpur, which he crossed in two directions; in the former region a continuous series was found to extend from the Cretaceous (covered by representatives of the Deccan traps) to the Pliocene. From 1877 to 1879, Blanford was chiefly engaged on office work in Calcutta, one part of it being co-operating with Mr. H. B. Medlicott, the Superintendent, in preparing the 'Manual of the Geology of India.' Then he returned to England on furlough, which was extended to two years to enable him to represent the Indian Government at the meeting of the Geological Congress in Bologna. Returning to India in the autumn of 1881, he undertook the survey of the country between the Punjab and Quetta, till he was prostrated by a severe attack of fever. This ultimately obliged him to return to England, where he arrived in April, 1882. Under medical advice he retired on pension after 27 years' service on the Indian Geological Survey, and though after a time he recovered his health, his constitution was less robust than in former days.

Early in 1883 he married Miss Ida Gertrude Bellhouse, and settled at Bedford Gardens, Campden Hill, converting a studio at the back into a library, which was adorned by trophies of the chase collected in India. Here three children—two sons and a daughter—were born, and the rest of his life was spent. He enjoyed good health on the whole, though occasionally liable to slight attacks of gout or bronchitis, but a year or so before his death these attacks became more severe, and signs of failing physical powers, happily unattended by mental weakening, began to make his friends anxious; but the end came more rapidly than they anticipated, for he passed away after a brief illness on June 23, 1905, his last literary work being a notice of his old friend and companion, H. B. Medlicott, contributed to these obituaries.

Release from official duties simply gave Blanford more time for the work which he loved, but Indian zoology now received more attention than geology, although the latter was anything but neglected. He was also frequently consulted upon survey matters by the authorities in England, and rendered them many services. He became Secretary to the Geological Society in 1884, was elected President in 1888, serving for the usual period of two years, and in 1895 was appointed Treasurer, holding the office till his death. In 1884 he presided over the Geological Section, when the British Association met at Montreal, and attended the Toronto meeting in 1897, afterwards joining the party to Vancouver Island. He took part in the meetings of the International Geological Congress at Bologna, Berlin, London, and Paris, served on the Councils of the Royal, the Geographical, and the Zoological Societies, and was a Vice-President of all. Before leaving India he had been a President of the Asiatic Society of Bengal, and when visiting Canada he received the degree of LL.D. from the McGill University, Montreal. The Geological Society awarded him their Wollaston Gold Medal, and the Royal Society marked its appreciation of the value of his Indian researches by conferring on him one of the Royal Medals in 1901. He received the Military Medal for the Abyssinian Expedition, the Cross of St. Maurice and St. Lazare in 1881, and was made a Companion of the Indian Empire as late as 1904.

Not many men have made more numerous contributions to scientific literature or bestowed more pains on what they wrote. His first book, 'Observations on the Geology and Zoology of Abyssinia,' was published in 1870. It begins with a written account of the expedition, and nothing could be better, considering the inevitable difficulties, than the scientific portions. He had succeeded in determining the true succession of the principal rock systems in Abyssinia and in defining more accurately their character, and he had collected zoological specimens, representing about 350 species of Vertebrata and 500 others, mostly Mollusca. His second book was the volume in the Report of the Persian Boundary Commission on the 'Zoology and Geology of Persia,' which maintains the high level of its predecessor and gives a very suggestive account of the physical geology of a region characterised by climatic extremes and a very low rainfall. The third was his contribution to the 'Manual of the Geology of India,' equal in bulk to rather more than one of the two descriptive volumes, a work the value of which is universally acknowledged, and the fourth in order were the volume on Mammals and two of those on Birds, contributed to the Fauna of British India, the whole of which series, 17 volumes, he edited for the Government of India.

His shorter papers, according to a list printed in the 'Geological Magazine,'* exceed 175. The first, published in 1854, was geological, a short notice of a section exposed in excavating for the West India Docks. The first zoological paper (the fourth of the series), written in conjunction with his brother, and published in 1860–1, was entitled "Contributions to Indian Malacology." The papers on the former subject mostly appeared in the publications of the Indian Geological Survey, the 'Quarterly Journal of the Geological Society,' and the 'Geological Magazine.' They cover a wide field, physical geology, stratigraphy and palæontology, but it will suffice to mention three of a more general character, characterised no less by breadth of view than by accurate knowledge of the subjects. The first, his Address to the Geological Section at Montreal, discussed the occasional conflict between the evidence for geological age derived from marine formations on the one hand and terrestrial (land and freshwater) on the other. The second, the Presidential Address to the Geological Society in 1889, contained a clear account and some valuable criticisms on the work done at the first and second meetings of the International Geological Congress; the third, the corresponding address for the next year, was "On the Permanence or otherwise of Ocean Basins." In this valuable contribution to a very difficult subject, Blanford exhibits the judicial character of his mind and adopts neither extreme, holding that while the general evidence in favour of the permanence of land areas and of ocean basins is very strong, there are certainly exceptions, where large tracts of the former have been submerged, and this for more than a thousand fathoms, and cases where portions of the latter, now at least that depth, have been raised above the sea to connect distant lands. His other writings show the same mental qualities, for, in the words of an anonymous writer, "His work in every department, whether in zoology, geography or geology, has been marked by thoroughness and accuracy."

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* Decade 5, vol. 2 (1905), p. 1. It goes down to 1901. I have made great use of this excellent article in drawing up the above notice, because I have been informed on good authority that a near relation of Dr. Blanford supplied many of the facts, and he himself revised the list of publications.

But if Blanford's death has been a loss to science, it has been even greater to his home circle and his many friends. Sound in judgment, free from prejudices or any angularities, with a gentle courtesy of manner and a desire to conciliate, nothing could make him depart from his own high standard of honour or the path which his conscience approved. He was, in short, a man emphatically to be trusted, for he exhibited in the daily work of life the calm courage which had carried him through the difficulties, and sometimes the dangers, of his earlier career, and while the last to seek his own advantage or magnify his own work, he was the first to recognise merit in that of others and ever ready to do them a service.

T. G. B.

THOMAS GEORGE BOND HOWES, 1853—1905.

Thomas George Bond Howes, more commonly known as George Bond Howes, was brought up in his boyhood at a private school. His education proper he would no doubt himself have attributed to Huxley, with whom he was for many arduous years associated at the Normal School of Science and Royal School of Mines as successively pupil, assistant demonstrator, and deputy professor. To Huxley's person and memory he was ever devotedly attached, and in 1895 he succeeded his celebrated master in the professorship of zoology, the duties of which he had been in fact performing for the preceding ten years.

While still Demonstrator of Biology at the Normal School, and at the same time Lecturer on Comparative Anatomy at the Medical School of St. George's Hospital, Howes published, in 1885, 'An Atlas of Practical Elementary Biology, with a Preface by Professor Huxley, P.R.S.' In this introductory notice Huxley explains that in 1872 one of his first cares had been the creation of a teaching collection for the use of his students, to contain, "in the first place, a series of preparations and dissections illustrative of every important fact in the structure of the animals and plants selected for study; and, in the second place, a corresponding series of drawings of the dissections, of large size and executed in such a manner as to facilitate the comprehension of the structures represented." In carrying out this important scheme, he candidly adds, "the whole burden of the work has fallen upon my former demonstrator, Mr. T. J. Parker (now Professor of Biology at Otago) and Mr. George Howes, who succeeded Mr. Parker, and now holds the office." He points out that, 'A Course of Practical Instruction in Elementary Biology,' published by himself and Professor Martin in 1875, needed supplementing by just such an atlas as Howes had supplied, "which, while starting from part of the work with which he has been occupied in our laboratory, contains so many accurate and well-devised additional illustrations that it will be hardly less useful to students who are engaged in the laboratory than to those who work independently of it."

In 1889 the course of elementary biology above mentioned was itself revised and extended by Howes in collaboration with his friend Dr. Dukinfield Scott, and in 1895 he edited and annotated the translation of Wiedersheim's 'Bau der Menschen.' Besides these services to scientific literature, his pen yielded some forty original contributions, of which a catalogue is given in the 'Proceedings of the Linnean Society' for the present year (1905). The longest and most important of the series is that which he wrote, in conjunction with Mr. H. H. Swinnerton, 'On the Development of the Skeleton of the Tuatara, *Sphenodon punctatus*, with Remarks on the Egg, on the Hatching, and on the Hatched Young.' Material for this treatise was supplied him with generous zeal by Professor Dendy, who used with great success his point of vantage for procuring it, while holding the chair of biology in the Canterbury College of the New Zealand University. Eggs of this interesting animal, which is supposed to be "the sole surviving representative of an early and widely ancestral amniote type," were brought over, with tender care, from New Zealand by Mrs. Dendy, and were incubated in the laboratory of the Royal College of Science. Three out of the six precious specimens ran the full developmental period. The young Sphenodons made good their own escape by help of the horny shell-breaker with which Nature has provided them, and led an observant and apparently contented life for three or four months. They then succumbed, it is thought, as occasionally happens with animals far higher in the scale of existence, to the effects of over-feeding or of feeding at a time when they ought to have been asleep. Hospitality was unthinkingly prolonged by their friends into the period which these lizards or lizard-like creatures should normally have spent in peaceful hibernation. Among the results arrived at by their elaborate treatise, Howes and Swinnerton consider that they have added "fresh testimony to the belief in the batrachian affinities of the living Sphenodon," while strengthening the demonstration of its chelonian resemblances and extending them to the Plesiosauria. "Sphenodon," they say, "judged from our standpoint, must be regarded as the surviving representative of that group of animals ancestral to all the living Sauropsida, and to at least the Dinosauria, Pterodactyla, and Ichthyosauria of the past, if not of the Mosasauria and Dolichosauria also, and unquestionably intimately related to the Anomodontia." They recall the view, much earlier advanced by Howes,* "that a sharp distinction may be drawn between the *coraco-sternum* of the Batrachia (*an archisternum*), and the *costal-sternum* of the Amniota (*a neosternum*), the existence of which in any Batrachian or Stegocephalian has not been proved." In the succeeding paragraph, they say, "And as to the skull, the now well-known fact that, whereas in the Amniota the hypoglossal nerve-bearing region, truncal in origin, is incorporated in the occiput, in the Batrachia these nerves are postoccipital," once again opens the gap between the Batrachia and Amniota; indeed, so markedly that "our ideas may be systematised by applying to the former condition the term *Archicraniate*, and to the latter that of the *Syncraniate*." Finally, they lead up to, but leave open, the question, whether terrestrial vertebrates are descended from batrachian reptiles or reptilian batrachians; that is, from a group which possessed a coraco-sternum and an archicranium, or from one which possessed a costal-sternum and a syncranium, "structural combinations which go hand in hand." Every page of this memoir carries evidence of the extremely careful investigation conducted by the joint authors. But also it is marked throughout by the distinguishing characters which Howes impressed on his own work. Acute and fearless criticism is frequent; equally or more frequent are expressions of admiring appreciation. At every point astonishing familiarity is displayed with the literature of the subject in all its bearings. The contributions of Germany and Japan, of Belgium and France, and Russia, of England and New Zealand and the United States of America, are all weighed in the balance. A paper is not neglected because it is of ancient date, nor yet because it has only been published "during the passage of this memoir through the press," or even shortly after the revisal of the proofs.

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* 'Nature,' vol. 18, p. 269, 1891.

As may be gathered from his writings in general, Howes found his favourite study in the anatomy of vertebrates. Nevertheless, when delivering addresses as President of the Malacological Society, in 1896 and 1897, he showed a commanding familiarity with all that had been written on the subject of Mollusca. His addresses as President of the South-eastern Union of Scientific Societies in 1900, and of Section D of the British Association in 1902, were equally impressive as the fruit of wonderfully capacious reading combined with high intellectual capacity. He was, however, by no means a voluminous author, although it might well have been expected that he should be by those accustomed to his voluminous fluency in expounding any topics which appealed to his easily kindled enthusiasm.

Howes was born on September 7, 1853, and may be said to have spent a quiet, unostentatious, and comparatively uneventful life in accumulating and dispensing vast stores of biological knowledge. He was elected a Fellow of the Royal Society in 1897. He was long the treasurer of the Anatomical Society, which he helped to found in 1887. From 1895 to 1903 he was the highly efficient zoological secretary of the Linnean Society. He served on the Council of the Zoological Society, and on its Reorganisation Committee, and was more than once a vice-president. In everything he undertook it was with him evidently only a question of doing the best work which the situation demanded or permitted; but it was seemingly without question that he undertook everything which he was asked to undertake. It is a sort of axiom in the intellectual world that a man may be killed by worry, but not by work.

In the case of Howes, his ungrudging readiness to serve was mated with an ambition to serve supremely well. It may seem unworthy to describe this noble eagerness as containing in itself the element of worry, and yet one can scarcely help believing that, as a result of too continuous effort, the brain at last gave way beneath the strain of its own restless activity. Early in 1903 his health broke down, and without any substantial recovery in the interval, he died on the 4th of February, 1905, in the fifty-second year of his age. He leaves a widow and daughter, colleagues, distinguished pupils, and many other warm friends, to deplore his loss. He was a man of religious temper, cheerful, affectionate, generous. As already explained, the energy of his life was given to science, but not to a lucrative branch of it. Had his life been prolonged, he might have secured fame by authorship; as it is, his reputation must largely rest on those whom he successfully trained in his lecture-room and laboratory, and who gratefully acknowledge the debt they owe to his wide learning, his skill as a teacher, his inspiring energy. There can be little doubt that he long rejoiced in the prodigal expenditure of his great powers, but in the concluding words of a stirring address, which he delivered in 1900, we may fancy a touch of prophetic pathos and self-allusion when he says, "Higher ambition than that of adding to the sum of knowledge no man can have; wealth, influence, position, all fade before it; but we must die for it if our work is to live after us."

T. R. R. S.

FERDINAND BARON VON RICHTHOFEN, 1833—1905.

Among the active promoters of geology and geography during the last half century, no one has filled a more distinguished place or has had a wider circle of friends and admirers all over the world, than the illustrious Professor in Berlin whose unexpected death on October 6 last sent a thrill of deep regret through every civilised country. Up to the last he retained so much of his youthful enthusiasm and power of work, that even those who knew him most intimately and could watch him from day to day, saw little sign of old age, and looked forward to a further continuance of his influence and assistance in the scientific progress of future years. But his end came in the midst of his labours. As he was working in his wonted place at his writing-table, a sudden seizure deprived him of speech. It left him, however, so fully conscious that an early recovery was hoped for. But a little later another stroke came while he was in a profound sleep, and after two days and a half he passed peacefully away without illness or suffering of any kind.

The family to which Baron von Richthofen belonged is one of some position in Silesia, where it owns extensive estates. Born at Carlsruhe in that country on May 5, 1833, he received his early education at the Roman Catholic gymnasium of Breslau, under ecclesiastics of whom he used in later life to speak with respect for their learning and their skill in imparting knowledge; yet he would refer regretfully to the monotonous restraints imposed by them which, inasmuch as they curbed free and strenuous intercourse among boys, had in his case, as he thought, induced a certain reserve and diffidence which he had in vain endeavoured to overcome. He would sometimes contrast his school experience with the very different training furnished by the public schools of England. From school he went to the University of Breslau, and then to that of Berlin, where he took his degree of Dr. Phil. in 1856.

Before completing his University career, he had already shown his strong bent towards the studies to which his future life was to be devoted. Well grounded in all the main branches of the natural sciences, it was in geological and geographical studies that he took specially absorbing interest. The writings of Leopold von Buch and Alexander von Humboldt appear to have had a decisive share in directing his course, and he spoke in later years with grateful enthusiasm of his indebtedness to these two great masters. Von Buch opened out for him the ample fields of geological research. The glowing pages of Humboldt kindled in him that eagerness for geographical exploration which carried him far and wide in the Old World and in the New. Perhaps it may have been the influence of the example of these two heroes which drew him at first into the study of ancient volcanic rocks; for Humboldt had begun his scientific career by writing a little treatise on the basalts of the Rhine, while Von Buch, fresh from the influence of Werner, had found in Landeck and in his early journeys into Italy proof of the aqueous origin which his master had so long claimed for all the basalts of the globe. Be this as it may, there can be no doubt that Richthofen, too, early felt the fascination of the problems presented by volcanic phenomena and ancient igneous rocks. He began, however, with a somewhat more adventurous flight into that difficult department of Nature than had been attempted by his two geological masters. He chose as the subject of his thesis for the doctorate a group of igneous rocks, on which he wrote a Latin essay, "De Melaphyro," and as soon as his University time came to an end, he started off on his first extended scientific investigation, for which he selected the region of Predazzo, St. Cassian, and the Seisser Alp. His observations on that area were begun in the summer of 1856, and were continued in subsequent years, but their publication did not take place until 1860, when they appeared in his remarkable monograph, wherein he arranged the sequence of the formations in that complicated region, discussed the igneous rocks and their successive epochs of eruption, worked out the tectonic arrangement of the several rocks, and propounded the view that the dolomite masses of the Tyrol represent ancient coral-reefs.

During the next three years he had ample opportunities of extending his knowledge and experience of field-work by journeys in the Northern Tyrol, Vorarlberg, North-Eastern Upper Hungary and Transylvania. His published papers of that period on the intrusive melaphyres of Moravia and on the trachytes of Hungary show how keen was his early interest in the tectonics and petrography of the igneous rocks, and at what a youthful period of his career he had thoughtfully considered the family relationships of these rocks, and, in particular, the important and independent character of the rhyolites. At Vienna he came into contact with the distinguished group of geologists and mineralogists who at that time flourished in the Austrian capital. Among these men he was more especially drawn to Franz Ritter von Hauer, afterwards for so many years the Director and guiding spirit of the Austrian Geological Survey. It seems to have been as a consequence of this cordial friendship that Richthofen was induced to become a volunteer on this Survey, and to spend four of the pleasantest and most profitable years of his life in hard systematic field-work among the Eastern Alps.

But his passion for geographical exploration in a wider and less-trodden field than an European mountain-range came at last to be gratified in a wholly unexpected way, whereby the current of his life was at once and permanently changed. In 1860 he had the good fortune to be chosen scientific member, with the rank of Secretary of Legation, in a Prussian Embassy, sent out to negotiate commercial arrangements with the native States of Eastern Asia. During this expedition he had an opportunity of spending some months in Japan, and of visiting Formosa, the Philippines, Java, and Siam. He did not, however, return with his comrades to Europe, but, fired with the desire of making extensive explorations into the heart of the Asiatic continent, he remained behind in the expectation of being able to carry out what had been a long-cherished dream. But, after some journeys in India and Cashmere, difficulties arising from the disturbed state of China arrested his further progress, so that at last, postponing his quest for some more favourable occasion, he crossed the ocean to California, with the view of studying the metalliferous and volcanic regions of the western border of North America. The full development of petrographical types of eruptive rocks in that remarkable territory soon kindled anew his enthusiasm for this department of geology, and furnished him with rich material for study. As the fruit of this research there appeared, in 1868, his now classic paper on the "Principles of the Natural System of Volcanic Rocks," which was published in English, in the first volume of the 'Memoirs' of the California Academy of Sciences. In this essay the relations, classification and order of sequence of the rhyolites, trachytes, propylites, andesites, and basalts were clearly expounded. A philosophical discussion was likewise given of the relations of modern to ancient eruptive rocks, of the origin of volcanic rocks, and of the connection between the distribution of these rocks and the configuration of the surface of the globe. One notable result of this residence in Western America was to enable the geologist to obtain such a mastery of the English language as is not often reached by a foreign man of science.

The design of Asiatic exploration, though necessarily postponed for a time, had never been laid aside. Richthofen discussed the subject with some of the American geologists, especially with J. D. Whitney, then in the height of his activity and usefulness as a geological pioneer in the Far West. At last the bold scheme was planned of making a prolonged geological survey of the interior of China. On August 3, 1868, Richthofen left San Francisco and recrossed the Pacific. He then began the series of journeys which, during a period of four years, extended over most of the provinces of the Celestial Empire. He made voluminous notes on the physical geography and geology of that vast region, and on the distribution and condition of its teeming human population. At the same time he formed an extensive collection of the minerals, rocks, and fossils of the various districts in which he sojourned. He was the first to reveal to the outer world the wide extent of workable coal in China. The commercial importance of his observations came to the notice of the European merchants in the East, and with the view of inducing him to continue and complete them, the expenses of his later journeys were partly defrayed by a contribution from the Shanghai General Chamber of Commerce, to which, in 1870, he forwarded a series of Reports on the economic and commercial aspects of the regions through which he had travelled. But the large amount of scientific material which he had amassed demanded years of patient elaboration.

He returned to Europe at the end of 1872, after twelve years of travel, and, settling in Berlin, set to work on the preparation of his monumental work upon China. The first volume did not appear until 1877; the second was published in 1882, the fourth in 1883, and the 'Atlas' in 1885. The third volume has never been issued, but it is to be hoped that the large bulk of material collected by the author for its completion may yet be given to the world. Instead of confining himself to the region actually traversed by himself, Baron von Richthofen discussed the general physical geography of Central Asia as a whole, and with great skill explained the broad configuration and grouping of the mountain chains of the region. Among the problems of which he presented a new solution, perhaps the most interesting and important was that of the Loess, which attains such colossal dimensions in the Celestial Empire. He brought forward a large body of evidence to show that this widespread and thick deposit is of Æolian origin, being due to the transport of dust by the winds. The fourth volume bears ample witness to his diligence and ability as a field-geologist and collector: it is devoted to the Palæontology of the scene of his labours, and though he entrusted the determination and description of his fossils to others, it is to his skill and enthusiasm that we owe the establishment of the existence of the Cambrian, Silurian, Devonian, and Carboniferous systems in China. He it was also who brought to light the Chinese development of the floras of Carboniferous, Jurassic, and Tertiary times.

Richthofen's geographical prowess was so well recognised, that in 1879, after the publication of the first volume of his 'China,' he became Professor of Geography in the University of Bonn. There he spent four happy years, during which he issued the successive volumes of that work. It was during this interval that he married Irmgard, daughter of Karl Freihern v. Richthofen, the gracious and accomplished lady to whom he had long been devotedly attached, and who became thenceforward his helpful companion in his work and in his journeys. As a teacher he soon showed that he possessed pre-eminent educational gifts, whereby he attracted students around him, kindled in them some of his own enthusiasm, and gave to geography a more defined and a higher place as a mental discipline in the studies of a University. His success in this sphere led to his being called, in 1883, to the Professorship of Geography at Leipzig. But all who had watched his career realised that before long he must inevitably be withdrawn to Berlin as the national centre where most scope would be found for the development of his schemes as a teacher and organiser of educational methods. The expected transference took place in 1886, when he became Professor of Geography at the metropolitan University of Germany. There he threw himself with all his characteristic originality and ardour into the task of devising and arranging such illustrations of geography as would bring vividly before the eyes and minds of his students, and of the general public, the characteristic features of land and sea, and the appliances for scientific exploration and travel. In connection with the University a Geographical Institute was organised by him, in which these various collections were arranged, and where ample space was found for large and small lecture rooms, map-drawing rooms, survey-practice rooms, a reference library, and large reading-room, well furnished with current geographical literature in all languages. In his later years he gave special attention to the illustration of the sea, and succeeded in prevailing on the authorities to build a Museum for Oceanography ("Meereskunde"), in which he sought to display, by help of charts, maps, models, paintings, diagrams, and apparatus, every feature of the sea which is capable of such illustration. Nor did he confine his efforts to a strictly scientific field: all that could help to convey a sense of the practical work of navigation and exploration, or quicken the popular interest in the navy, came within the scope of his aims. He journeyed through the maritime countries of Europe in search of the best examples of illustration for his purpose—models of ships of all ages, methods of ship-building, and types of intruments employed in navigation. For spaciousness and even luxuriousness of accommodation, for breadth of conception and for the completeness with which the designs have been carried out, there is probably no geographical establishment in the world that can equal the Geographical Institute of Berlin, planned and carried out by the genius and unwearied enthusiasm of its Director.

For many years he had taken keen interest in the welfare of the Geographical Society of Berlin, and when he returned to the capital as his permanent home, he had an opportunity of devoting his practical energies to its improvement. He introduced several reforms in its organisation, and communicated to it so much of his own personal activity and enthusiasm as eventually to place it in the distinguished position which it now holds among the geographical institutions of our time.

Beginning his career with geological studies in the field, Baron von Richthofen passed into geographical investigation with the inestimable advantage of a training in detailed observation and induction. He thus became a geographer of the highest type. To him the mere addition of so many hundred square miles of territory to what was already known of the earth's surface, and the opportunity of affixing the names of friends and benefactors to peaks, promontories, rivers, and inlets were matters of no moment; it was the grand features of land and sea that interested him, their origin, their history, their relations to each other, their influence on the progress and destiny of mankind. His geological apprenticeship with the Austrian surveyors among the Eastern Alps, and his early researches in mountain-structure and the behaviour of igneous rocks, could hardly fail to give him that grasp of physical features, combined with that knowledge of detail which are so often lacking in travellers and explorers. But besides his scientific accomplishments he possessed in rare measure the personal qualities which go so far to ensure the success of an explorer—health and strength alike of body and mind, a wide range of natural knowledge, courage, patience, endurance, tact, and kindliness. It may have been the consciousness of the possession of these qualities, combined with a recollection of the pleasure which their exercise had given him in his varied wanderings in Europe, America, and Asia, that led him, in response to many requests, to give a course of popular lectures on scientific travel. These lectures he subsequently extended, and in 1886, the year of his transference to Berlin, amidst all his University and other work, they appeared as his admirable 'Führer für Forschungsreisende.' No one but a born and trained explorer, who had enjoyed ample experience by flood and fell all over the globe, could have written such a volume, so full of the ripest practical knowledge, so broad in its conception of what exploration should be, and so clear and emphatic in its statement of the accomplishments which are needed for the making of a successful traveller. Every department of research is luminously presented in his chapters, which may be regarded as a contribution of the first importance to geology and physical geography. Among the shrewd pieces of advice in the book the author recommends the explorer to travel alone, with only the attendants necessary for his purpose, but without any friend or companion who would do more than look after the material well-being of the expedition. Various treatises with similar aims had been given to the world before the publication of Richthofen's volume, from the time of Ami Boué's 'Guide du Géologue Voyageur' onwards, but none of them so thoroughly deserves to be put into the hands of every man who proposes to undertake the examination of new or little-known regions, and who is willing to learn beforehand what is expected of him by those competent to judge.

Baron von Richthofen, as a familiar and welcome participator in various International Congresses, and an honorary member of many of the learned societies of Europe, was one of the most widely known and most respected men of science of his day. His tall figure and courtly presence made him conspicuous wherever he came, while his quiet, earnest and kindly manner irresistibly attracted those with whom he came in contact. His home at Berlin, where he was greatly aided by the full intellectual sympathy of his devoted wife, became a centre to which geographical pilgrims from all lands made their way. With his students he was not merely the learned professor, but the personal friend who guided and helped them while they were with him, and watched with keen interest their subsequent progress when they left. The 'Colloquium' which he established among them afforded ample opportunity for that friendly social intercourse between teacher and taught which is so valuable an aid in the higher education. This personal influence he cultivated so ardently that perhaps few modern teachers have equalled him in the enthusiastic devotion of his pupils. On his sixtieth birthday his old students at Bonn, Leipzig, and Berlin offered him as their most welcome gift a 'Festschrift' of geographical essays, in evidence that, of the seed which he had sown so widely, some had taken good root. While his original contributions to science will always keep his name fresh as one to whom geology and geography stand greatly indebted, it is probably his educational work in the organising and developing of geography as a mental discipline in university training that has had the most influence in his own lifetime. But this influence could never have been so far reaching and profound had it not been based on the recognition of his place as one of the great scientific travellers of the nineteenth century. He leaves a blank in the ranks of science which none of his survivors can wholly fill, and his memory will be warmly cherished by all who knew him as one of the ablest, worthiest, and most lovable of men. A. G.

FREDERICK WOLLASTON HUTTON, 1836—1905.

Captain F. W. Hutton, whose name has been so long and so intimately connected with the Natural History of New Zealand, died at sea on October 27, 1905, while on his way back to the Colony after a short stay in England. He was born in 1836, at Gate Burton, Lincolnshire, of which village his father was Rector. At the age of fourteen he was a midshipman on a ship trading to Calcutta, but he soon left the sea, and became a student at King's College, London. Before the age of twenty he received a commission in the 23rd Royal Welsh Fusiliers, and soon saw active service in the Crimea, and afterwards in India during the Indian Mutiny, where he was present at the Relief of Lucknow and in other engagements. His bent for science was already showing itself in various ways, and in 1860, on his return to England, he was elected a Fellow of the Geological Society, and gained considerable practical acquaintance in that science with the officers of the Geological Survey in Hampshire and the Isle of Wight. Fortunately for New Zealand, Captain Hutton, in search of fresh fields of work, turned his attention to that Colony, and, retiring from the army, arrived there in 1866. Soon he became attached to the Geological Survey of New Zealand, and commenced that series of researches into the geology of the Colony which lasted, with some breaches of continuity, throughout his life. In 1871 he was appointed Assistant Geologist, and removed from Auckland to Wellington; in 1873 he became Provincial Geologist of Otago and came to Dunedin, and before long, in conjunction with the late Professor Ulrich, he published an important work on the geology of Otago.

But Captain Hutton was not a man of one science only. During this time he had already been working also on the zoology of New Zealand, and when the position of Provincial Geologist lapsed with the abolition of the provinces in 1876, he was appointed Professor of Natural Science at the Otago University, and had charge of the Otago Museum, which had been designed and built under his device and direction, and which still contains so much evidence of the work that he did at that time. About four years afterwards he removed to Christchurch as Professor of Biology at Canterbury College, and soon began to instil into his students some of his own enthusiasm for natural science, as he had already done in Dunedin. Here, while continuing his researches on geological and zoological subjects, he found it necessary, for the sake of his students, to take up the study of botany, and with his natural clearness of insight and ability to grasp readily the essential details of a fresh science, he soon made himself familiar with his subject, and a little pamphlet which he published on the structure of the common weed "shepherd's purse' received favourable notice at the time.

He continued to occupy the chair of Biology at Canterbury College till the death of Sir Julius Von Haast, when he became, in addition, Lecturer on Geology, and had temporary charge of the Canterbury Museum. Soon after, on the resignation of Mr. Forbes, he was permanently appointed Curator of the Museum. Thereupon he resigned his professorship of Biology, but continued to lecture on geology till about three years ago, when he found the strain of lecturing in addition to his museum work pressing too heavily upon him.

For nearly forty years he had been constantly engaged with his various official duties, and had never revisited England, but early in the year 1905 he was granted six months' leave of absence, and he left New Zealand in March, full of anticipation of the pleasure he would have in renewing old acquaintances and of meeting personally the many scientific friends whom he already knew so well by correspondence; but owing to a breakdown in his health soon after his arrival in England, these pleasurable anticipations were only in a very slight degree realised.

Such, then, is a brief outline of what may be called Captain Hutton's official life; but this only covers a small part of his activity: during these busy years he found time to prepare and publish a long list of papers and works embodying the results of original research, which he contributed to the scientific journals of New Zealand, Australia, and Europe. Many of these, as already stated, deal with geology, but a far larger number are devoted to zoology, and they cover practically every class of animals from mammals to protozoa, and it can safely be said that no other New Zealand zoologist will ever be able to cover so wide a field. Indeed, Captain Hutton's work has made this unnecessary, and at the same time impossible. When he arrived in New Zealand he found its zoology practically untouched; some of the birds and more conspicuous animals had, indeed, been collected and described by various scientific expeditions that had visited New Zealand; but even of these little was known, and all the others were unnamed and unknown. To the task of describing these, Captain Hutton set himself with determination and enthusiasm, and not only did he possess the necessary knowledge and skill required for the commencement of the work, but he had also the rare faculty of self-development with his work, and of acquiring new methods of research and investigation as they were needed.

From his pen there came in rapid succession descriptions and catalogues of fishes, mollusca, crustacea, worms, echinoderms, sea-anemones, and insects of all kinds. Many of these appeared in the 'Transactions of the New Zealand Institute,' to which he has been a constant contributor from the commencement, and many of his catalogues were issued as separate publications. As the result of his labours, aided to some extent by that of his students and others, whom he started and encouraged in the same task, the preliminary work of naming and classifying the animals of New Zealand has been to a great extent completed, and it is a great satisfaction to all zoologists that he was able, with the assistance of several co-workers, to publish in the early part of 1904 an 'Index Faunæ Novæ-Zealandiæ'—*i.e.*, a complete list, with references of all animals hitherto recorded from New Zealand. A survey of the pages of this work will give some idea of the number of the animals that were described by himself, while the numerous congratulatory letters on the completion of this work that were publicly presented to him at a meeting of the Philosophical Institute of Canterbury in May, 1904, bear eloquent testimony to the value placed upon his work by those best qualified to judge. More recently, under the title 'Animals of New Zealand,' he has published a beautifully illustrated account of the air-breathing vertebrates of New Zealand.

But Captain Hutton was not content with merely naming and describing new species: no one knew better than he that this work, valuable though it was, formed only the preliminary statement of the problems presented by the phenomena of animal life, and these problems he never lost sight of. To the particular problems of the origin of the New Zealand fauna and flora he specially devoted himself, and brought to bear upon it all his wide knowledge as a zoologist and his experience as a geologist. His contributions to the solution of this complex problem have made his name known throughout the world as a recognised authority on all matters connected with the geographical distribution of animals and plants. It was from their bearing on this question that he took such a keen interest in the results of the various Antarctic expeditions that have recently returned from their fields of observations, and even after his arrival in England, he contributed an important article to 'Nature' on the Antarctic continent.

But he attacked even wider questions still. One of his very first contributions to scientific literature was a review of Darwin's 'Origin of Species' in the 'Geologist' in 1861—a review which gained from Darwin an appreciative letter, of which Captain Hutton was justifiably proud—and throughout his career Captain Hutton returned from time to time to the more philosophical questions underlying the whole of natural science. Some of his articles on these subjects were issued a few years ago in book form under the title of 'Darwinism and Lamarckism.' A later work, 'The Lesson of Evolution,' which contains his presidential address to the Australasian Association at Hobart in 1902, and kindred essays, attracted much attention not only from naturalists but from thinking men in general.

Captain Hutton naturally took a keen interest in the welfare of the various scientific societies in New Zealand. As honorary secretary and president of the Otago Institute in years past, he did much to establish it on a firm and permanent basis. During the whole of his stay in Christchurch he was a most active member of the Philosophical Institute of Canterbury, acting as secretary, treasurer, and on several occasions as president. He was secretary of the Australasian Association for the meeting in Christchurch in 1891. Later on he was president of Section D (biology), and in 1902 he was president of the Association itself, on the occasion of its meeting in Hobart. On the retirement of Sir James Hector as manager of the New Zealand Institute, about three years ago, Captain Hutton was prominent amongst those who promoted a Bill for the reconstitution of the Institute, providing for the better representation of the various district Institutes on the Board of Governors, and for an elected president, and when the new constitution came into force in January, 1903, he was by common consent elected as first president under the new constitution—a position he still held at the time of his death.

As a teacher he was singularly clear and original in his methods of exposition, and possessed the valuable faculty of arousing enthusiasm in his pupils, several of whom have become regular contributors to various departments of natural science. Though he was not a public man in the ordinary sense, he took his full share of public duties in all matters where he could contribute special knowledge without becoming involved in questions of local politics. Being thoroughly sincere, open, and straightforward in all his actions, he was a keen and unsparing critic of anything that bordered upon pretence and humbug, but to all genuine students and workers he was a warm friend and a ready helper, and the loss of his valued advice and ripe judgment will be felt by many.*

* For this Obituary Notice the Royal Society is indebted to Professor Charles Chilton, M.A., D.Sc., F.L.S., Canterbury College, Christchurch, New Zealand.

GEORGE BOWDLER BUCKTON, 1818—1905.

In his address to the Royal Society in November, 1905, the then President, Sir William Huggins, reported among the year's losses the death of George Bowdler Buckton, who "to the deep grief of a large circle of friends, passed away at the ripe age of 88, leaving behind him a noble example of unwearied devotion to the successful prosecution of scientific work, notwithstanding great physical infirmity."

Most men who attain to scientific eminence find the study of a single branch of science, or of one or two allied branches, sufficient to occupy a lifetime. It is comparatively seldom that the same man is able (like Buckton) to pursue with success two such dissimilar studies as Chemistry and Entomology. There was also another unusual feature in his scientific career. Most men who occupy themselves with Entomology and kindred pursuits do so chiefly in their younger days, and more or less abandon the study in later life, whereas Buckton, though always interested in Natural History, devoted his earlier years to Chemistry and Physics, and the latter half of his long life to the preparation of his valuable works on Entomology.

Born in or near London, on May 24, 1818, and brought up at Oakfield, Hornsey, Buckton was the eldest son of a large family. His father, George Buckton, was Proctor of the Prerogative Court of Canterbury, and his mother, Eliza, was the daughter of Richard Merricks, of Chichester, Deputy Lieutenant of the County.

At about the age of five years, Buckton sustained an accident which permanently crippled him, a misfortune which he bore throughout life with courage and silence, but which must have been peculiarly painful to a man of his large build and energetic temperament. Nevertheless, it is probable that if he had been able to lead a more active life, he would not have had the same opportunities of carrying on his scientific pursuits so steadily and successfully.

As Buckton's infirmity unfitted him for public school and University life, he received tuition from the Rev. Oliver Lodge, Rector of Barking, and the Rev. Dr. Meuse, formerly Headmaster of the Cholmondeley School. He was popular with his class-mates, shared their escapades, and was often carried pick-a-back by some stronger comrade on daring excursions. Necessarily left much to his own resources, he became a good classical scholar and an extensive reader. He also devoted himself successfully to music and painting, which remained his favourite recreations throughout life. He showed much ingenuity in the construction of appliances to illustrate his youthful lectures on scientific subjects.

While still scarcely more than a boy, Buckton made the acquaintance of Thomas Bell, who proved his close friend for more than 40 years, and by whose encouragement he became an earnest student of Natural History, beginning to form collections and make observations on bees, butterflies, shells, bats, birds, etc., round London and elsewhere. Most of the birds he collected were shot and stuffed by his own hand; he also became a good fisherman.

Astronomy was another of Buckton's favourite studies, and, being always alive to the interest of collateral issues in science, he started quite early grinding telescopic specula. Frankland, with whom he afterwards became intimate, vied with him in being the first English physicist to produce specula on glass, which method had been lately inaugurated by the French philosopher Foucault. Subsequently, Buckton worked specula of 12 inches diameter, and, as an amateur, mounted them equatorially.

On the death of his father, he moved into London, residing at Queen's Gardens, W., where he built himself a circular observatory on the leads of his house, and made more than one telescope, using his own brass turnings, etc. About this time he became the pupil, friend and assistant of Professor A. W. von Hofmann (of whom, it will be remembered, Sir William Perkin and other distinguished men were also pupils), at the Royal College of Chemistry. His first recorded scientific paper was "Observations on the Deportment of Diplatosomine with Cyanogen," published in the 'Journal of the Chemical Society' (vol. iv, pp. 26 to 33), and subsequently republished in translation, in several French and German periodicals in 1851 and 1852. A succession of other papers followed, among the most important being those relating to his discovery and isolation of the radical, mercuric methyl, etc. ('Proc. Roy. Soc.,' vol. ix, 1857 to 1859, pp. 91, 92, 309 to 316; 'Phil. Trans.,' 1858, pp. 163 to 169; 1859, pp. 417 to 435). The last of the series, worked out with the collaboration of William Odling, was upon aluminium compounds, and was published in 'Proc. Roy. Soc.,' vol. xiv, 1865, pp. 19 to 21. A full list will be found in the Royal Society's Catalogue of Scientific Papers.

Meantime Buckton had been unobtrusively carrying on his Natural History studies, his earliest recorded papers on the subject being "On the Application of Cyanide of Potassium to Killing Insects" ('Zoologist,' 1854, vol. xiv, pp. 436 to 438) and "Notice of several species of Bats captured in England during the present autumn" ('Proc. Linn. Soc.,' vol. ii, 1854, pp. 259 to 261).

In 1845, Buckton became a Fellow of the Linnean Society, serving in 1855–6 upon its Council, and coming into contact with Yarrell, Westwood, Saunders, Huxley, Hooker, and other naturalists. In 1852 he joined the Chemical Society, and in 1857 the Royal Society, when he became a member of its Philosophic Club, the meetings of which he attended with great interest, till extreme old age compelled him to relinquish them. In 1883 he became a Fellow of the Entomological Society. He served on the Councils of all these Societies from time to time. He was also elected a Member of the Entomological Society of France; the Academy of Natural Sciences at Philadelphia, etc.

In 1865, Buckton married Mary Ann, the only sister of his friend Professor Odling, of Oxford. He purchased the estate of Weycombe, at

Haslemere, Surrey, where he built himself a stone-gabled house, according to his own designs, taking with him his observatory and transit instruments. Here he lived a peaceful, happy life for the remainder of his days; and out of eight children, five daughters and a son survive him; likewise his widow.

After settling at Haslemere, although Buckton kept his private laboratory and lathe room, and gave private lectures on Chemistry and Physics to his children and friends, he devoted himself chiefly to Natural History, commencing by studying Parthenogenesis in Aphides, which led up to the first of his valuable series of Entomological works, his 'Monograph of British Aphides,' published by the Ray Society in four volumes, from 1876 to 1883. The numerous illustrations to this work were made under the camera lucida and lithographed and coloured by his own hand.

Continuing the study of the somewhat neglected sub-order Homoptera, he published his 'Monograph of British Cicadæ or Tettigidæ' (2 vols., Macmillan) in 1890–1891, in which he was assisted by his children, who collected specimens and coloured many of the plates under his direction. This was followed by the 'Natural History of Eristalis Tenax; or, the Drone Fly' (1 vol., Macmillan, 1895); and finally by a large and important work on the Membracidæ of the World, issued by Lovell Reeve & Co. in 4to. parts from 1901 to 1903, with many illustrations. A supplement to this work was published in the 'Transactions of the Linnean Society' in July, 1905, only two months before the author's death. The original plates of the Monograph of Membracidæ have been presented to the Hope Museum, Oxford.

Buckton's deftness of hand in arranging his hundreds of microscopic slides was remarkable; often in laying out the delicate nervous organisation of an insect, he would make use of the sting of a wasp, as the finest available instrument. He was in constant communication with various scientific societies at home and abroad, and carried on a large correspondence with the unfailing energy and courtesy that reminds one of an earlier period, when letter-writing was a fine art; this was distinctly perceptible even in his business letters.

Buckton instructed his children himself till they were 9 or 10 years of age in a variety of subjects, from Grammar and Languages to Euclid, Drawing and Physiology. He had an unusual gift of exposition, and was always ready to share his knowledge with others. He often gave tools or specimens to small boys who showed interest in Natural History.

In spite of his infirmity, the charm of his personality deeply impressed even strangers casually meeting him for the first time; and a friend writes, "None who ever met him could fail to be struck with his kindly courtesy, his intense vitality, his wide range of knowledge, and his unflagging interest in every topic affecting mankind."

In his earlier days, Buckton travelled in Italy, France, and elsewhere, saw the Commune, and even climbed the barricades, his great will-power enabling him to surmount obstacles that might have daunted stronger men.

The Observatory which Buckton took with him when he removed from London to Haslemere was erected in the grounds at some little distance from the house; but his visits to it were discontinued after a serious accident that befell him in 1882, when he overbalanced himself in trying to reach the long focus of a Newtonian. He lay with a leg fractured in two places for some hours before he was found; and his recovery, though complete, was slow.

Buckton interested himself sympathetically in all sorts of local matters, acting as Treasurer and Chairman on various local bodies. In politics he was a Conservative; in religion a moderate Churchman, with broad sympathies, and he gave liberally to Church and Schools. Among his large circle of friends he numbered many of eminence, among whom were Tyndall and Tennyson, of whom, as they resided not far from Haslemere, he saw a good deal.

Though sometimes swift and uncompromising in his judgments, and naturally of a quick temper, he was nevertheless gifted with remarkable self-control, especially as regarded his physical infirmity. Those most intimately associated with him can recall no single instance of moodiness or murmuring; but when some unexpected hindrance presented itself, he would allude, half-sadly, and half-humorously to his disability; a reference not easily forgotten by those who heard it. It has been remarked that "the most striking thing about him was his magnificent calm."

Buckton kept his remarkable powers of work and energy to the last. The finely-executed bust by R. Hope-Pinker, exhibited in the Academy of 1904, plainly indicated how little old age had impaired his clear intellect and vigour. He was finishing some water-colour sketches of Norway up to within a few weeks of his death.

Buckton succumbed at last rather from the natural exhaustion of old age than from any physical disease. He sustained a chill, followed by three weeks of illness and suffering; but he was conscious to the last, and his spirit passed peacefully away on the night of September 25, 1905, in the presence of his wife and children.

After cremation, the ashes were buried, according to his wish, in Haslemere Churchyard, in the presence of many relatives, friends, and members of the various Societies to which he had belonged. At the time the village was celebrating the subdued festivities of its Harvest Home, adding an appropriate calm and beauty to a somewhat unusual ceremony.

The present Lord Tennyson has written, concerning Mr. Buckton, "Truly a devoted, spiritual, knightly nature, with a faith as clear as the height of the pure blue heaven. His views and my father's upon Life, Death and Immortality were very much alike. My father used to say, 'My most passionate and most earnest desire is to have a fuller and clearer knowledge of God.'" There is scarcely a preface to any of his larger works in which Buckton does not incidentally reveal his deep-lying interest and trust in things unseen.

W. F. Kirby.

CHARLES BARON CLARKE, 1832—1906.

Charles Baron Clarke was born at Andover, Hampshire, on June 17, 1832. He was the eldest son of Turner Poulter Clarke, J.P., and Elizabeth Parker. His father was the son of Turner Poulter Clarke and Elizabeth Baron; his mother was the daughter of James Parker and Elizabeth Ward. One of his grand-uncles, Charles Baron, an enthusiastic gardener, was the founder of the Agricultural Society of Saffron Walden. Baron's sister, Clarke's paternal grandmother, shared her brother's love of plants; three of her sons owed largely to her influence a taste for scientific enquiry. These were Clarke's uncles, Joseph, a careful antiquarian, who founded the Saffron Walden Museum; Joshua, an able field botanist and a recognised authority on the flora of Essex; and Benjamin, a systematic botanist of decided originality, spoken of by Bentham as "one of our most careful observers." Clarke shared the tastes of two of his uncles; he took, in addition, a deep interest in social science. The latter taste he seems to have inherited from his maternal grandfather; his mathematical powers appear to have been derived from his father and his paternal grandfather.

As a boy, Clarke was sent to the preparatory school of Mr. Tomlinson, at Salisbury. From this he went to King's College School, London, where he passed through the higher forms and then attended classes in the College before proceeding to Cambridge. He entered at Trinity in 1852; graduated B.A. and was bracketed Third Wrangler in 1856; was elected a Fellow of Queen's in 1857; was called to the Bar at Lincoln's Inn in 1858, and appointed Mathematical Lecturer of his College in the same year; and took the degree of M.A. in 1859. He continued a resident Fellow of Queen's till December, 1865, when he was appointed to the Bengal Education Department.

On reaching India in 1866, Clarke was at first attached to the Presidency College, Calcutta, but was soon appointed Inspector of Schools in the Eastern Division of Bengal, with his headquarters at Dacca. Early in 1869, Dr. T. Anderson, Superintendent of the Royal Botanical Garden at Calcutta and of Cinchona Cultivation in Bengal, was invalided to Europe; on Anderson's recommendation Clarke was appointed to officiate in both posts. Towards the end of 1870, Anderson died in Scotland, but Clarke was not relieved of his duties in the Botanical Department till July, 1871, when, on the arrival of Dr. (now Sir) G. King, who had been appointed Anderson's successor, he was able to revert to his substantive appointment at Dacca. In 1874, Clarke was transferred to the Presidency Division of Bengal, with his headquarters at Calcutta, and in 1875 he was again transferred, as Inspector of Schools, to the Northern Division, with his headquarters at Darjeeling.

In 1877 Clarke came to England on two years' furlough; when this expired he did not return to India, but was placed on special duty at Kew, to assist Sir J. D. Hooker in preparing the 'Flora of British India.' This deputation continued till 1883, when Clarke reverted to duty in Bengal as Inspector of Schools in the Presidency Division, and temporarily as a Professor in the Calcutta Presidency College. In December, 1884, Clarke was appointed to officiate as Director of Public Instruction in Bengal, and in March, 1885, his services were placed at the disposal of the Government of Assam, where he served as Inspector of Schools, with his headquarters at Shillong, till his retirement, at the age of 55, in June, 1887.

On his return to England, Clarke settled at Kew, to be near the Herbarium at the Royal Gardens, where he worked without a break as a volunteer till within a few days of his death, which took place, after a short illness, on August 25, 1906.

The influence of Mr. Tomlinson on Clarke's intellectual development seems to have been considerable, and the foundations of the interest which he took in geographical and historical studies appear to have been laid at his preparatory school. At King's College School he was much impressed by the teaching of Mr. Hann, a man of marked individuality, who was mathematical master.

As an undergraduate at Cambridge, Clarke, from his King's College connection, was one of a circle of close friends which included John (afterwards Sir J.) Rigby, a Member of his College and subsequently Second Wrangler in Clarke's year, and Henry Fawcett, also of the same year but a Member of Peterhouse. Clarke, who, both as an undergraduate and as a resident Fellow, was Fawcett's especial intimate, had not known the latter, or only knew him by sight, in his school days. Though a native of Salisbury, Fawcett did not attend the same preparatory school; Fawcett was, besides, a year Clarke's junior, and though they were contemporaries at King's College School, they were not class fellows. But from Easter, 1851, till they went to Cambridge, both attended the same mathematical lectures at King's College, and during this period began their lifelong friendship. The migration of Fawcett to Trinity Hall brought him and, as a consequence, his friend Clarke, into contact with Leslie Stephen, who had also been at King's College. As a result, Stephen was drawn into their circle and became a lifelong friend of both. Characteristic of the members of this brilliant coterie was an interest in discussions bearing on political economy and a foible for unconventionality in matters of dress. In the case of Clarke the interest continued to the last; the foible became second nature.

That a group of thoughtful undergraduates should take pleasure in the discussion of intricate social problems is, fortunately, not unusual; nor is it surprising that the feelings of youth should often induce the adoption of advanced views. The outlook, however, not infrequently alters when the realities of life are faced and the discussion of such problems are too frequently left to the professional philanthropist and the party politician, whose interpretation of economic truths is occasionally unconsciously affected by a natural enthusiasm or subconsciously influenced by a thirst for place. Clarke was neither, and his views on political economy were therefore particularly refreshing. He treated the subject as one of the exact sciences, and if the sanity of his outlook seemed at times almost cynical, those who knew him knew also that this was to be attributed to the subject and not to the man. For the factors in a social problem never presented themselves to his mind as mere abstractions, and any approach to cynicism was foreign to his kindly nature.

While in residence at Queen's, the penchant for what was then his favourite study incidentally led to his taking an active part in the Cambridge Election of 1863 which Fawcett unsuccessfully fought. He was still more active in his friend's behalf a year later, when Fawcett first stood for Brighton. Between these two contests occurred the more memorable one, so vividly described by Leslie Stephen, into which Clarke and Stephen flung themselves with almost unacademic ardour, that resulted in Fawcett's appointment to the Chair of Political Economy at Cambridge. Clarke's intervention in these struggles was due to his personal affection for Fawcett, whose views on social problems were substantially his own. The relative position of the two was, however, far from being that of disciple and teacher. Though not a good public speaker, as Fawcett was, Clarke's conversation was easy and, from his store of facts, he was remarkably ready in discussion. We learn from Leslie Stephen that Fawcett made frequent use of Clarke's often pithy expressions on economic subjects. Where his interests were shared, whether his views were accepted or not, political economy was a frequent topic in Clarke's letters and talk. Besides various pamphlets and letters on special problems, some of these written at the request of Indian officials with reference to legislative measures that were being contemplated or enforced, in which his views are stated with lucidity and directness, Clarke has recorded his attitude towards the subject as a whole in his 'Speculations from Political Economy,' published in 1886.

The circle at Cambridge to which Clarke belonged was mainly a mathematical one, but most of its members seem to have held the view that the value of a mathematical training lay chiefly in its excellence as a branch of intellectual gymnastics. It is not clear that this was precisely Clarke's view; the fact that he published no purely mathematical paper suggests that he may have shared it. He was, though not technically a musician, fond of good music, and while in India was interested in native, particularly Bengali, music. The interest in this case was, however, mathematical rather than æsthetic, and that he was attracted to this side of the art as a whole may be deduced from his note on the "Equal Temperament of the Scale" which appeared in 'Nature' in 1883.

The members of this Cambridge circle had a house in the Lake Country where they could stay during vacations. Here Clarke spent most, if not all, of the Easters of his time at Queen's; Leslie Stephen has recorded how, during Clarke's last visit, Easter 1865, he and Clarke made an ascent of the

Pillar Rock in Wastdale. Clarke, always a traveller, during long vacations went further afield. In the earlier years he visited Scotland, once going as far as Skye; in 1862 he visited Madeira; several visits, usually as one of a climbing party, were paid to the Alps, the last of these being made in 1865. Mountain adventure was not, however, the only object of his journeys; his private herbarium shows that during all of them he was an assiduous botanical collector. While engaged in his favourite occupation in Madeira, his inexplicable presence on a lonely hillside and the aberrant nature of his attire led to his being arrested as an escaped prisoner.

Clarke's most sustained botanical work while he was a resident Fellow of Queen's was done, however, in England and chiefly in his own country, Northern Hampshire. The results were embodied in a list of the flowering plants of Andover, his birthplace; this list, completed before he left for India, was printed at Calcutta in 1866, shortly after his arrival there. The modest title and remote origin of this first contribution to what was now to be his favourite science failed to conceal the independence with which the subject had been treated, and the trenchant criticism which it evoked led to a reply which showed that in Clarke the reviewer had to deal with no mean antagonist.

The work of an Indian Inspector of Schools involves much touring during a considerable part of the year, and affords opportunities for a study of the flora of the country traversed hardly to be surpassed. Of these opportunities Clarke took full advantage during his service in Bengal and Assam, supplementing them as occasion offered by vacation visits to districts and provinces other than those within which his duties lay. During the first two years and a half of his Eastern service Clarke travelled extensively in East Bengal, where the physical conditions are such that the only practical mode of conveyance is by boat. The considerable collections he had amassed, and the bulk of his other belongings, were lost, early in 1868, by the wreck of the country boat which for the time being he had practically made his home; his own safety was due to his being a powerful swimmer. Undiscouraged, he began afresh, and besides collecting largely in his own division, was able to visit the Khasia Hills, Tipperah and Chittagong. He occasionally complained that, owing to the pressure of official and administrative duties at the Botanical Garden, he was able to do "less real botanical work during a year of his superintendentship than he could accomplish in a month of his travelling appointment." Still his temporary connection with the Cinchona Department gave him an opportunity of studying the flora of temperate Sikkim, enabled him to pay his first visit to the high Himalayan passes, and led incidentally to his making a journey in Madras and the Nilgiris. His transfer to Calcutta in 1874 permitted him to partially explore the Sundribun forests of the Gangetic Delta, to visit Chutia Nagpur and to make a holiday tour in the Punjab Himalaya. When Darjeeling became his headquarters he was enabled to make three more tours in Alpine Sikkim, to investigate temperate British Bhutan, to study the flora of the North Bengal plain, and to make a holiday journey which lasted three months and a half, his most sustained purely botanical excursion, in Kashmir and on the Karakoram Range. The collections accumulated during these various journeys amount to 25,000 numbers, representing about 5000 species. The whole he made over to Kew when he reached England in 1877.

When Clarke returned to India in 1883 he made a second and more extended tour in Chutia Nagpur, and in the protected native States to the south-west, paid botanical visits to various portions of Central Bengal and explored subtropical Sikkim and the submontane forests of the Terai and the Duars. His transfer to Assam in 1885 enabled him to renew and extend his acquaintance with the flora of the Khasia Hills, to explore the valleys of the Brahmaputra from Dhubri to Sadiya, and of the Surma from Silhet to Silchar, as well as to visit the Naga Hills and Manipur. The collections amassed during Clarke's second period of service in India amount to some 10,000 numbers; a complete set of the specimens was presented to Kew after his retirement in 1887.

In the course of his Indian career Clarke was able to acquire, at first hand, a knowledge of the Indian flora comparable in extent with that of Hamilton, Wallich and Hooker, and only surpassed by that of Griffith. Clarke was an admirable field botanist; his commissariat and equipment when travelling were Spartan in their simplicity; his physique was such that he seemed to regard the ascent of a Himalayan slope or the passage of a tropical rice-swamp much as he regarded an English geological excursion. His indifference to climate was such that his clothing did not vary from what he wore in England, whether he was on a Himalayan pass in early spring or in the Gangetic plain in the hot season. His specimens were always carefully selected and prepared. The precise and often full field notes, frequently accompanied by serviceable analyses, were always made in the field; every gathering received a separate serial number, so that misquotation of a plant collected by Clarke is impossible; energetic and tireless, he always dealt with the specimens of a particular date before he retired to rest, no matter how long or trying the day's march might have been. A feature of Clarke's work as a collector was the especial attention given by him at particular times to individual natural families. His notes show that from 1866—69 his interest was great in *Commelynaceæ*, from 1869—71 in *Cyrtandraceæ*, from 1871—73 in *Compositæ*, in 1874 in *Gentianaceæ*, from 1874 onwards in *Glumaceæ* generally, but after 1876 more particularly in *Cyperaceæ*, the family as to which in later years he was to become the recognised authority. Another characteristic of Clarke's work was the comparative indifference with which he regarded trees: as a collector of herbaceous species he has, perhaps, never been surpassed. These two peculiarities were more marked during the first phase of his Indian career than they were during the second, which began after he had spent six years continuously at work at the Herbarium at Kew.

Clarke's contributions to botanical literature during his first ten years of service in India include monographs of the *Commelynaceæ* and *Cyrtandraceæ* of Bengal, published at Calcutta in 1874, and of the Indian *Compositæ*, also published there in 1876; with six minor papers in the Linnean Society's 'Journal' between 1870 and 1876. In 1874 he further earned the gratitude of Indian botanists by reprinting, with a preface at his own cost, Roxburgh's 'Flora Indica,' which had become scarce and hardly procurable. While on furlough during 1877—79, and on deputation at Kew during 1879—83, his additions to botanical literature were more extensive. They include accounts of over fifty of the natural families described in the second, third and fourth volumes of Sir J. D. Hooker's 'Flora of British India'; three minor papers in the 'Journal of Botany'; ten in the Linnean Society's 'Journal'; and one, an important review of the ferns of Northern India, in that Society's 'Transactions.' During this period he also wrote two of the volumes, that on *Commelinaceæ*, issued in 1881, and that on the *Cyrtandreæ*, issued in 1883, of De Candolle's 'Monographiæ Phanerogamarum.'

The family *Cyperaceæ* was, as we have seen, the last of a series of families which had proved especially attractive to Clarke. This particular interest did not become dominant till 1876, near the close of his first period of Indian service; it continued so, however, during the rest of his career. While helping forward at Kew, between 1877—83, the preparation of the 'Flora of British India,' he had little time to study his favourite family till shortly before he left England; his first papers on the subject are two of the minor contributions in the Linnean Society's 'Journal,' already alluded to, which appeared in 1883. But during his two last months in England advantage was taken of his special knowledge in having the Indian species of *Cyperus* rearranged. When Clarke was leaving for India, Sir J. D. Hooker asked him to publish the results of his work, so as to aid later on in dealing with the genus in the 'Flora.' On reaching India, Clarke similarly rearranged the rich Indian material of the genus in the Calcutta Herbarium; he thereupon published, in the Linnean 'Journal' for 1884, an important review of the Indian species of *Cyperus* in both herbaria.

Clarke's only botanical contributions during his second residence in India were notes on three of his botanical journeys, published in the Linnean 'Journal.' During this period, however, he studied and rearranged the Calcutta material of the order *Cyperaceæ*, and when he reached Kew in 1887, after having critically examined and distributed the contents of his second Indian Herbarium, he devoted the greater part of his time to a detailed study of the *Cyperaceæ* of the globe, his object being to complete a general monograph of this difficult family. As his work progressed he gradually became the recognised authority on the subject, to whom botanists of every nationality sent their collections from all parts of the world for identification. The results of his work in this direction are to be found in numerous papers published in many periodicals at home and abroad. The *Cyperaceæ* in most of the principal collections in Europe were also submitted to him for critical examination and determination. In addition he wrote, at the request of Sir J. D. Hooker, the account of the *Cyperaceæ* for the 'Flora of British India,' published in 1893—94, and at the request of Sir W. T. Thiselton-Dyer the accounts of the same family for the 'Flora Capensis,' published in 1897—98; for the 'Flora of Tropical Africa,' published in 1901—02; and for the 'Index Flora Sinensis,' issued in 1903—04. His exhaustive acquaintance with the family was turned to further account in defining, in a paper which appeared in the 'Philosophical Transactions' in 1893, the relationship that natural biologic regions, so far at least as botany is concerned, bear to the tabulation areas which are most useful in dealing with the distribution in space of living organisms. He utilised his familiarity with *Cyperaceæ* a second time in 1898 in defining the limits of the sub-subareas that comprise the tabulation area of British India. But his monograph of the family as a whole, though completed at the time of his death, remains still unpublished.

Clarke did not, however, confine himself exclusively to his favourite family during the period from 1887 onwards. Besides publishing the results of his Manipur journey, in the Linnean 'Journal,' he prepared for Sir W. T. Thiselton-Dyer accounts of three natural families for the 'Flora Capensis,' and of five for the 'Flora of Tropical Africa'; of two for Sir G. King's 'Flora of the Malayan Peninsula'; and of seven or eight for Schmidt's 'Flora of Koh Chang.' One of the last pieces of work he did was to finish Lowe's 'Flora of Madeira.' This is still unpublished; the text has been completed, but a memoir of Lowe on which Clarke was engaged when overtaken by his last illness remains unfinished.

Clarke possessed a considerable general knowledge of Natural History, but was not minutely acquainted with any special branch, and he rarely discussed zoological subjects. His acquaintance with Geology was more extensive, and though he seldom spoke at geological meetings, he frequently attended the excursions of the Geologists' Association, not only the shorter day or half-day meetings, but the longer excursions made by the members at Easter and Whitsuntide, and in August, and was always ready to enter into the discussion of such geological questions as arose during the excursions. He was hardly an active fossil collector, and his interest inclined rather to those wide problems in Geology where the science approaches the domain of Physiography. He was frequently able to intervene in discussions on the origin of river-systems, the formation of valleys, and similar topics, and to describe, from his Indian experience, cases very much in point. The extent and accuracy of his knowledge of Geography are incidentally shown in those papers which deal with the distribution of plants and are more directly seen in the various geographical text-books written by him for Macmillan between 1878 and 1894. Equally extensive was his knowledge of History generally and of English History in particular, though, if a conclusion might be drawn from his conversation, his interest was greatest and his knowledge most detailed with regard to the periods of the Tudors and the Stuarts. Unfortunately, none of the results of his historical studies have been published; for some time before his death he was engaged in writing a History of England and had completed the manuscript to the reign of James I. But the subject which, after Political Economy and Botany, most interested him was, perhaps, Ethnology. His knowledge of this science was extensive and his views were independent. He, however, published only one ethnological paper, on the Stone Monuments of the Khasi Hills, in the 'Journal of the Anthropological Institute' in 1874.

In Clarke were united all the qualities that gain esteem and hold affection: Open and sincere, he was neither prone to praise nor ready to blame. The peculiar charm of his manner seemed only to increase with years and longer intercourse. Mental powers which excite admiration were in him combined with singular modesty. Unselfish to a degree and unfailingly kind, he was always ready to place his time and knowledge at the service of others. Hospitable to a fault, the warmth of his welcome was only equalled by the difficulty with which he could be persuaded to accept the hospitality of others.

Clarke became a Fellow of the Linnean Society in 1867 and of the Geological Society in 1868. He joined the Council of the Linnean Society for the first time in 1880 and so served during sixteen years; he was a Vice-President seven times and was its President during two years, 1894–96. In 1882 he was elected a Fellow of the Royal Society and served on its Council during 1888—90. In 1897 he joined the Geologists' Association; in 1903, in conjunction with Professor Judd, he acted as Director of one of its excursions.

D. P.

LIONEL SMITH BEALE, 1828—1906.

Lionel Beale was born in the year 1828, in the parish of St. Paul's, Covent Garden. His father was a surgeon, and was one of the earliest Officers of Health in London. While a medical student at King's College, Lionel Beale showed talent as well as industry, particularly in the study of chemistry and anatomy; and before he had taken his degree he was invited by Professor Acland to take the part of anatomical assistant in the Natural Science School at Oxford. He remained there for two years, when he returned to King's College, and was succeeded by Victor Carus, of Leipzig. In 1852 Beale established a laboratory where he taught "the use of the microscope," and gave courses of instruction in physiological chemistry, and minute anatomy, normal and morbid. In 1853 he was appointed Professor of Physiology in King's College, Huxley being one of the candidates.

During these early years of his scientific life, Beale received guidance and help from Dr. Todd, Mr. Bowman, and Mr. Sambrooke. Between 1851 and 1858 he seems to have published nearly one hundred papers, most of them illustrated by his own skilful hand.

In 1859 he married Frances, only daughter of the Rev. Peyton Blakiston, M.D., F.R.S., and took the lease of 61, Grosvenor Street, which he retained until 1904. In a small greenhouse, and on a flat roof at the back of this house, he, for many years, grew orchids, ferns, and bulbs with skill and success. In the year 1882 he found it necessary to live in the country on account of his wife's health, and took a small cottage in a pine wood at Weybridge, travelling to and from London daily. His garden became well known amongst those interested in this pursuit, for he grew successfully palms, Japanese plants, and bulbs. One of his friends there was the late Mr. G. F. Wilson, whose wild garden at Wisley has now come into possession of the Royal Horticultural Society.

In 1892 his wife died at Weybridge, and this loss and the constant fatigue of railway-travelling began to affect his health. In 1896 he had an attack of apoplexy, which laid him up for several weeks—his first serious illness. In the year 1904 he returned to London to live with his son and daughter-in-law in Bentinck Street, where his general health markedly improved, although senile changes became more evident. In March, 1906, he had another attack of cerebral hæmorrhage. He became unconscious on the next day, and passed peacefully away on the 28th. He was then 78 years old. He was buried in Weybridge Cemetery, as was his wish, and in the presence of his near relations and a few of his oldest friends. One of his sisters, Miss Sophia Beale, survives him, well known for her sketches at home and abroad; and he leaves one son, Mr. Peyton Beale, F.R.C.S.

Dr. Beale was a self-contained man, at least in his later years, of somewhat less than the middle height, with good features and a pleasant smile. He was remarkable for scarcely showing signs of his age until his last illness, and always gave the impression of a busy and happy man, enjoying his scientific work and his garden with almost equal constancy. His old friend, Mr. H. T. Wells, R.A., painted an admirable portrait which will faithfully transmit his features to a distant day.

Dr. Beale's reputation was early established as a histologist, an original observer, and a skilful investigator, while his beautiful drawings added greatly to the value of his anatomical papers and of both his completed volumes on the same subject. He was admitted to the Royal Society in 1857,* and gave the Croonian Lectures in 1865 on the termination and destruction of the ultimate nerve-fibres.

In 1871 the College of Physicians awarded him the Baly medal, a distinction shared by him with Sharpey, Claude Bernard, Carl Ludwig, Brown-Sequard, Heidenhain, Burdon-Sanderson, Michael Foster, Maurice Schiff, Gaskell, Schäfer, Pavy, Langley, and Pavloff. The College also appointed him Lumleian Lecturer in 1875, when he chose for his subject "Life and Vital Action in Health and Disease."

As a lecturer, he was clear, accurate, and instructive; as an examiner, patient, and sound in judgment. Strongly as he held his own views, he never judged a candidate by his opinions, but by an impartial estimate of his knowledge and ability. As a physician, he was courteous, shrewd, and practical; as a colleague, honourable, and friendly.

Apart from his distinction as a practising physician, a demonstrator, and lecturer, Beale deserves lasting reputation as a histologist. His first investigations were on points of animal chemistry: the absence of chlorides in pneumonia, 'Medico-Chirurgical Trans.' (1852), and the methods of identifying glycose in the urine (1853). Then came observations on the minute anatomy of the liver ('Phil. Trans.,' 1856). His methods of soaking tissues in dilute acid solutions led to valuable results in tracing the course of ultimate nerve-fibres, and his staining with carmine was a fruitful method of investigation. When he began his investigations, the admirable work of Bowman on the histology of renal structures and of muscular fibres was the most striking advance in this country, and Beale's close relations with Dr. Todd and Mr. Bowman were of great importance. He also had the merit of distinguishing between the structure Protoplasm or, as he called it, Bioplasm, which forms the nucleus and body of a cell, from the formed material or intercellular structure without power of movement or growth or reproduction. The one he called "germinal matter," the other, "formed material."

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* Copy of extract from Certificate of Dr. Lionel Smith Beale, February 4, 1856: —

The author of (1) a paper on the "Minute Structure of the Liver," ordered for publication in the 'Phil. Trans.' for the present year; (2) a paper on the "Absence of the Chlorides in the Urine in cases of Pneumonia," published in the 'Medico-Chirurgical Transactions'; (3) a work on the 'Microscope, and its Application to Clinical Medicine,' 1854.

Distinguished for his acquaintance with the sciences of Physiology and Pathological Chemistry.

The title of a very early paper of his was "An Attempt to show that every Living Structure consists of Matter which is the seat of Vital Actions, and Matter in which Physical and Chemical Changes alone take place."

The discovery and description by Schleiden and Schwann of the structure and essentially identical properties of vegetable and animal cells led directly on to the observations of Goodsir and Redfern in this country, and to the cellular pathology of Virchow. Beale's researches were in the same direction, and established the distinction between cellular and intercellular or connective tissues.

It was, perhaps, unfortunate that the difference of view held by Beale from that maintained by Huxley and many others in this country and abroad was formulated as the question between "vitalism" and "materialism." The truth does not always lie between two extremes. If we invoke the "vital principle" to explain every difficulty, we so far put a bar to the advancement of science. But when we have traced all vital functions to the primordial living ovum, and "Nature's self untwisted lies into its first consistencies," the untwisted knot is as hard to be "explained" as ever. As Du Bois-Reymond said, *Ignoramus et Ignorabimus.*

In estimating Dr. Beale's work, we must remember the great importance of a new method. The differential staining of tissues was the method he introduced, and ultimately his views on the nucleus and the cell were based on the reaction of animal and vegetable nuclei and protoplasm to carmine and other discriminating agents. He left no school behind him, but the influence he had on his contemporaries was important. His two works on histology, "The Microscope in Clinical Medicine," published in 1854, and "How to Work with the Microscope," published in 1857, covered to a large extent the same ground. The former is a careful description not of the instrument itself, but of needles, scalpels, scissors, methods of staining and mounting sections, chemical reagents, and of drawing, measuring, injecting, and staining. The second part is not a laboratory handbook, but a histological account of tissues and fluids of the body, both normal and morbid, including the histology of tumours and of the vegetable and animal parasites then known. It is well illustrated by drawings of the author's selection and execution.

Simultaneously with the successive editions of 'The Microscope in Medicine' appeared not less numerous issues of 'How to Work with the Microscope.' It was dedicated to the writer's old friend, Mr. Sambrooke. The first part is devoted to a description of the instrument and its accessories, and is followed by a systematic account of the structures—animal, vegetable, and mineral—revealed by the compound microscope. As in the earlier volume, not only are the construction of the microscope and its accessories fully illustrated by the author's drawings, but numerous woodcuts are also collected at the end of the volume figuring the structure of human tissues.*

A third volume, of very different character, was published many years later, and reached a second edition in 1882. It is a reprint of lectures on medicine at King's College, and is specially occupied with "Slight Ailments," with no reference to histology or carmine. It is of purely practical value, and by its many shrewd observations shows a power of observation at the bedside that no one would have expected who was only acquainted with his histological works.

In his later life Dr. Beale wrote chiefly in defence of vitality as a cause of living phenomena. He held strong convictions on its application to metaphysics and theology, and on these subjects was much influenced by the late Dr. Thring, of Uppingham, and Dr. Wace, the present Dean of Canterbury.

Professor Osler writes as follows of Beale's reputation in the United States and Canada ('Lancet,' April 7, 1906):—"The influence of Dr. Beale as a scientific investigator and a clinical physician was much more widespread than perhaps was recognised in London or Great Britain at large. In part, at least, the unfortunate terminology was responsible for the unpopularity of his views in some quarters. Both in Canada and the United States there are scores of men of my day who, like myself, knew Dr. Beale only by his writings, who will hear of the death of their old teacher with sincere regret, and who will recall with gratitude labour which often helped to lighten their own."

Titles of Some of the most Important Scientific Papers of Dr. Beale.

His first book was on "The Microscope and its Application in Practical Medicine: a Course of Lectures, 1854."

1853. "On the Absence of Chlorides from the Urine in Cases of Pneumonia." 'Med. Chir. Trans.'

1854. "The Microscope in Clinical Medicine."

1856. "On the Anatomy of the Biliary Ducts." 'Phil. Trans.,' published separately, with sixty photographs of the author's drawings.

1857. "How to Work with the Microscope."

The two books were slightly varied in title in successive editions and overlapped.

The fourth and last edition of "The Microscope in Medicine" was published in 1878.

The fifth and last edition of "How to Work with the Microscope" was published in 1880.

The Croonian Lecture delivered before the Royal Society of Physicians, "On Life and Vital Action in Health and Disease," show that every structure consists of matter which is the seat of vital actions and matter in which physical and chemical changes alone take place.

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* Both books were modified in title in successive editions.

1862–3. "Nerve-endings in Voluntary Muscles." 'Proc. Roy. Soc.,' 1862–3.
"Minute Anatomy of Sarcolemma and Striped Muscle," 1864. 'Quart. J. Micr. Sc.'
1863. Paper on "The Branching of Nerves in the Frog's Bladder." 'Phil. Trans.,' 1863.
1864. "Termination of Nerve-Fibres." *Ibid.*
1864. "Minute Anatomy of Sarcolemma and Striped Muscle." 'Proc. Roy. Soc.'
1865. He delivered the Croonian Lecture before this Society on "The Ultimate Nerve-Fibres Distributed to Muscles and some other Tissues."
1865. "The Minute Structure of Dentine." 'Quart. J. Micr. Sc.'
1869. "Minute Structure of Papillæ of Frog's Tongue." 'Quart. J. Micr. Sc.'
1870. "Bioplasm." *Ibid.*
1871–2. "Nerves of Capillary Vessels." *Ibid.*
1880. Presidential Address to the Royal Microscopical Society.

Dr. Gustavus Mann has favoured the writer with the following appreciation of Dr. Beale's histological work:—

"Looking back from our present knowledge, Beale's greatest achievement is to have recognised that a distinction must be drawn between the 'germinal' and the 'formed' matter of tissues. This view, first published in 1861, in "The Structure of the Simple Tissues," was adversely criticised by Max Schultze in "Das Protoplasma," because 'it lay outside the cell theory.' How little justified this dictum was has since been established, for Beale's germinal matter includes what we now call the nucleus and also cytoplasm in an active state of metabolism. Beale was led to his conception by the different behaviour of tissues towards his ammoniacal carmine solution. He wrote 'all elements of fresh tissue which stain readily with carmine, alone possess the power of growth and of producing matter like itself out of material differing from it entirely in properties and powers. I therefore called it germinal or living matter to distinguish it from the formed material. The latter may possess remarkable properties and may undergo various physical and chemical changes under the influence of heat, moisture, oxygen, etc. It may permit some fluids to permeate it, and may interfere with the passage of others. It may contribute to the stability of the organism, and perform a variety of important functions, but it cannot take the place of the germinal or living matter, nor in many cases does it exhibit its characteristic properties after the death of the germinal matter belonging to it.' As examples of 'formed' material, he mentions the white fibrous tissue of tendon, the matrix of cartilage, and the body of red blood corpuscles. These far-reaching conclusions were not mere speculation, but were the legitimate outcome of a very careful study of the changes which the body, and particularly the connective tissues, undergoes during its development from an embryonic to the adult state; of the changes met with in fully-grown individuals wherever there is a replacement of old tissues by new, as in the skin; and, finally, of the alterations produced in normal tissues as the result of infection, both local and general. In his "Disease Germs," many experiments are quoted in which the marked proliferation account of the white blood corpuscles is carefully depicted, and although we now know definitely that white blood corpuscles are not the agents producing infectious diseases, the idea underlying Beale's theory is correct, for in the light of Ehrlich's recent researches there is no doubt that a cancer cell, *i.e.*, Beale's germinal matter, may have its virulence greatly increased under new conditions of environment.

"Beale did not fall into the common error of considering histology from a purely anatomical point of view. He saw that in physiological research, physics, anatomy, and chemistry must go hand in hand. By the first, the physical phenomena may be elucidated. By the second, the minute mechanism concerned in these phenomena is ascertained. By the third, the nature of the chemical analyses and syntheses taking place in the living organisms are to be determined. How much Beale valued the chemical aspect of minute anatomy is shown by the care he devoted in his text-book to micro-chemical methods, and by the labour he expended on urinary deposits, the numerous figures being drawn with the greatest possible care, and, above all, drawn to scale. He further recognised the acid reaction of the 'germinal' matter and the basic properties of 'formed' matter.

"To him also belongs the credit of having for the first time practised the method of fixing tissues by injection, for experience had taught him how rapid are the alterations ensuing after death if due precautions be not taken to prevent *post-mortem* changes. Among Beale's discoveries the distribution of the peripheral nerves and the minute structure of the cells of both the central and peripheral systems are especially worthy of mention. Working with the simplest methods, but gifted with the greatest patience and a natural aptitude for microscopical research, he discovered that the sympathetic ganglion cells of the frog were connected with two distinct processes, one of which, the straight one, was continuous with the central part of the cell, while the other or others were arranged spirally round the straight fibre and were joined to the circumference of the ganglion cell. We now know that Beale's spiral fibre is in reality the termination of a nerve-fibre springing from another cell and carrying stimuli to the ganglion cell. His methods of examining tissues in dilute acetic acid allowed him to see delicate nerve-fibres scarcely less numerous than those revealed by Ehrlich's *intra-vitam* staining with methylene blue. It is only natural that Beale, having shown by his simple method that the distribution of nerve-fibres in striped muscle is so abundant, should have treated Cohnheim's gold method for nerve-endings and the results which Kühne obtained by this method and Max Schultze's tetroxide method, sceptically. His conception of the distribution of the nervous system was that there are no free nerve terminations; that 'in all cases the terminal distribution of nerves is a plexus, network, or loop, and hence that in connection with every terminal nervous apparatus there must be at least two fibres, and that in all cases there exist complete circuits, into the formation of which central nerve-cells, peripheral nerve-cells, and nerve-fibres enter.' This view of Beale has been revived to a great extent by Apathy, and Pflüger has recently expressed his doubts as to the correctness of Waldeyer's neuron-theory; but if we may place reliance on Ehrlich's *intra-vitam* staining with methylene blue, and on the various modifications of Golzi's method, there cannot be any doubt that nerve-fibres have definite terminations, and that Beale's conception of 'circuits' is not tenable. But though there be no direct continuity between efferent and afferent nerves of the cord, there is an indirect continuity by way of the tissues which are supplied with 'motor' and 'sensory' nerves. An equilibrium can only be established by both motor and sensory reflexes.

"Beale was partly led to his view by the important discovery that the nerve cells in the central nervous system of man, the dog, and cat, show a distinct fibrillation on being treated with dilute acetic acid. Not only did he trace these fibrils from the dendritic processes to the axis cylinder process, but also from one dendron to other dendra. As far as the arrangement of nerve-fibrils in nerve cells of the spinal cord is concerned, no new facts have been added to what Beale published 43 years ago, although by recent methods nerve-fibrils can be made plain to those who have no special gifts for microscopic work.

"Microscopical researches were to Beale a labour of love, and all who study his directions, how to become proficient in this line of investigation, cannot fail to be drawn unconsciously to follow the paths Beale travelled, and to endeavour to achieve as much as he did."

P. H. P-S.

PROCEEDINGS OF

THE ROYAL SOCIETY.

SECTION B.—BIOLOGICAL SCIENCES.

On Opsonins in Relation to Red Blood-cells.

By J. O. WAKELIN BARRATT, M.D., D.Sc. Lond.

(Communicated by Dr. C. J. Martin, F.R.S. Received August 11,—Read November 22, 1906.)

(From the Lister Institute of Preventive Medicine.)

In a previous communication* it was pointed out that opsonins, whose existence in relation to bacteria is generally recognised, are also produced by injecting foreign red blood-cells into the peritoneal cavity. The latter variety of opsonin, which is thermostable, not being destroyed by exposure to 58° C. for 30 minutes, is taken up by red blood-cells of the kind used for injection. Such red blood-cells, after being thus "sensibilised" by opsonin, are ingested by leucocytes.

Since it appeared obvious that the occurrence of phagocytosis might, under suitable conditions of experiment, be made use of in order to discover if a serum were capable of sensibilising red blood-cells, that is, if it contained opsonin corresponding to the red blood-cells employed, it was determined to ascertain if, proceeding along the line of investigation thus suggested, a method for the estimation of red blood-cell opsonins could be devised. It was further decided to study the nature of the action of such opsonins upon red blood-cells.

Estimation of Opsonin.

In attempting to make use of phagocytosis for detecting the presence of opsonin in serum a serious difficulty is at the outset encountered owing to the occurrence of spontaneous phagocytosis. When the red blood-cells of the rabbit, for example, are injected into the guinea-pig, opsonin for such red blood-cells develops in the latter's serum, which (after destruction of complement (alexin) by heating for 20 to 30 minutes at 58° C.*) may now be used to sensibilise the red blood-cells of the rabbit. If the sensibilised red blood-cells are added to the leucocytes of the guninea-pig, contained in the (heated) normal serum of the guinea-pig, extremely active phagocytosis of the red blood-cells at once takes place. If, however, sensibilisation is omitted, phagocytosis is still observed, though in considerably diminished degree. Attempts to overcome the disturbing effect of this residual or "spontaneous" phagocytosis have not as yet met with a sufficient degree of success to enable estimations of opsonin to be carried out in such cases.

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* J. O. W. Barratt, "The Phagocytosis of Red Blood-cells," 'Roy. Soc. Proc.,' B, 1905, vol. 76, p. 524.

There is, however, another class of cases in which spontaneous phagocytosis does not occur. Leucocytes contained in the serum natural to them do not ingest the red blood-cells with which they are normally associated.† If, for instance, the red blood-cells of the guinea-pig are injected into the abdominal cavity of the rabbit and the serum of the latter is employed for sensibilisation, the leucocytes of the guinea-pig, contained in (inactivated) guinea-pig serum are now seen to ingest such sensibilised red cells, though if sensibilisation is omitted no phagocytosis whatever occurs.

A serum of this kind was prepared from a rabbit, which will henceforth be referred to as Rabbit A. The serum was used for the series of experiments recorded in the tables.

Rabbit A.—Weight, 2300 grammes. Intraperitoneal injections were made as follows:—

On the 1st day the red blood-cells of 7·5 c.c. of guinea-pig's blood.
On the 11th day the red blood-cells of 4·5 c.c. of guinea-pig's blood.
On the 21st day the red blood-cells of 4·5 c.c. of guinea-pig's blood.

On the 118th day the rabbit was bled to death. The serum obtained was inactivated by heating to 58° C. for 30 minutes.

An attempt was first made to ascertain if opsonin could be removed from the serum of Rabbit A by the washed red blood-cells of the guinea-pig, added in varying amounts and acting upon the serum (diluted and undiluted) for periods of time ranging from five minutes to five hours (Table I). During this time the mixture of serum and red cells was continually shaken, either by hand or in a motor-driven shaker. The mixture was then centrifugalised and the supernatant liquid pipetted off (great care being taken to ensure the complete removal of the red blood-cells).

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* Otherwise hæmolysis of the red blood-cells occurs.

† All the experiments with sensibilised red blood-cells, given in the tables, were accompanied with control experiments on normal red cells. In these, as in numerous other control experiments not referred to here, the results as regards phagocytosis were invariably negative.

The washed red blood-cells of 0·005 c.c. of guinea-pig's blood were next added to 1 c.c. of the deopsinated serum, and the whole agitated for two hours at 37° C. The sensibilised red cells, after being washed in 0·85 per cent. sodium chloride solution, were added in excess to the leucocytes of the guinea-pig in inactivated guinea-pig serum and kept on a warm stage (37° C.) for two hours. The leucocytes were then examined under the microscope and the percentage containing red blood-cells noted, 2000 to 4000 leucocytes being enumerated. The percentages thus obtained are only approximately correct,* as will be readily understood by reference to Table III, but by making a number of tests under conditions as nearly as possible identical in respect of every detail, fairly concordant results are obtained.

The results, given in Table I, showed that it was possible to remove opsonin from the serum employed, provided that a sufficient number of red cells were used. After the red blood-cells of one or more parts of guinea-pig's blood had acted upon two parts of the serum of Rabbit A, the serum had completely lost its power of sensibilising red cells; in other words, it no longer contained opsonin in recognisable amount. If the red blood-cells of one part of guinea-pig's blood were shaken with four parts of serum, sufficient opsonin was usually left in the serum to sensibilise red cells; this was especially the case when the period for deopsination was short. In Table I no indication of the degree to which opsonin is removed is afforded beyond the fact that a period of five minutes is too short to effect complete removal at 37° C.

The enquiry now presents itself, how much serum is required to sensibilise the red blood-cells of a given volume of guinea-pig's blood? To investigate this point the series of observations recorded in Table II was undertaken.

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* The degree of phagocytosis obtained, apart from the degree to which sensibilisation of the red cells has been carried, depends chiefly upon the uniformity and the closeness of distribution of leucocytes and red cells. To secure the maximum degree of phagocytosis the cells must be evenly distributed and the leucocytes must not be separated by more than a few cell breadths from the erythrocytes, for leucocytes do not appear to recognise the presence of the latter until their processes come in contact with them. Difficulty was occasionally experienced owing to the occurrence of some degree of agglutination of the red cells after treatment with the rabbit's serum; separation could usually be effected by shaking, provided the proportion of serum employed was not too great (*i.e.*, $\frac{B}{S}$ not above $\frac{1}{20}$, compare Table III). The condition of the leucocytes also affects the degree to which the red cells are taken up. If a sufficiently extended series of tests are made the condition of the leucocytes tends to become the same in each experiment.

Table I.—Deopsination of Serum of Rabbit A, by means of the Red Blood-cells of the Guinea-pig. Temperature of Experiment, 37° to 38° C.

No. of expt.	Dilution of serum.	$\frac{B^*}{S}$.	Phagocytosis.† [The duration of the deopsinating action of the red blood-cells on the serum is given in brackets.]									
			[5 min.].	[10 min.].	[20 min.].	[40 min.].	[1 hr.].	[1½ hr.].	[2 hr.].	[3 hr.].	[4 hr.].	[5 hr.].
1	1 in 8	$\frac{8}{1}$							—			
2	Undiluted	$\frac{4}{1}$							—			
3	1 in 8	$\frac{2}{1}$							—			
4	Undiluted	$\frac{1}{1}$							—			
5	,,	$\frac{1}{1 \cdot 6}$				—	—		—			—
6	1 in 8	$\frac{1}{2}$							—			
7	1 in 2	,,			—		—				—	—
8	1 in 8	$\frac{1}{4}$							—			
9	Undiluted	,,							—			
10	,,	,,	+ + +		+ +			—		—		
11	1 in 8	,,	+ + +		+ + +			—		—		
12	Undiluted	,,	+ + +		+ + +			+ + +		+		
13	1 in 8	,,	+ + +		+ +			+ +		+		
14	Undiluted	,,	+ + +		—			—		—		
15	1 in 8	,,	+ + +		+ + +			+ + +		+ + +		
16	Undiluted	,,	+ +		+ +			+		+		
17	1 in 8	,,	+ + +		—			+ + +		+ + +		
18	Undiluted	,,			—			+ +		+ +		
19	1 in 8	,,			—			—			—	
20	,,	,,	+ + +	+ + +	+ + +	+ + +						
21	,,	,,			+ +	+ +						

* The washed red blood-cells of guinea-pig's blood (B) were added to the serum of rabbit A (S) in the proportions given. Thus, $\frac{B}{S} = \frac{8}{1}$ means that eight parts of guinea-pig's blood and one part of the serum of the rabbit were taken.

† In order to test phagocytosis, the red blood-cells of 0·005 c.c. of guinea-pig's blood were sensibilised with 1 c.c. of the deopsinated serum, diluted or undiluted, and then added to leucocytes obtained from the guinea-pig. The sign — means that no phagocytosis occurred; + that 0·2 per cent. to 0·7 per cent. of the leucocytes contained red blood-cells; + + that 1 per cent. to 1·5 per cent. contained red cells; + + + that the maximum degree of phagocytosis was obtained (2 per cent. to 5 per cent., in one instance 10 per cent., in another 20 per cent.).

In these experiments the red blood-cells of gradually diminishing amounts of guinea pig's blood were added to a given volume of the serum of Rabbit A, and the whole shaken for two hours at 37° C., the red cells being then washed and tested as to their power of exciting phagocytosis. It will be seen that so long as the ratio of the former to the latter exceeds 3 : 1 the red blood-cells are quite insufficiently sensibilised and fail to excite phagocytosis when added to the leucocytes of the guinea-pig. When the proportion is 1 : 1, phagocytosis becomes marked,* and no further increase in the amount of serum employed causes any recognisable augmentation of the degree of phagocytosis obtainable. From this it may be concluded that, as far as phagocytosis is concerned, this ratio represents complete sensibilisation. This does not, however, represent the maximum amount of opsonin which can be taken up, for it appears, from Table I, that the red blood-cells of one part of guinea-pig's blood in some of the experiments removed opsonin to such an extent from four parts of serum that the serum was no longer capable of sensibilising red blood-cells, though in other experiments sufficient opsonin for this purpose was still left. An explanation of the difference in the amount of opsonin taken up in the two cases is probably to be found in the circumstances that phagocytosis is essentially a surface phenomenon depending upon the degree to which the outer part of the red blood-cell is sensibilised, and no advantage arises from the opsonin acting upon the cell in its whole thickness.

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* The highest degree of phagocytosis obtainable was found to be that in which 2 to 6 per cent. of the leucocytes had taken up red cells at the end of 2 hours at 37° C. (after 2 hours little or no increase occurred). This range represents the variation to be expected under the most favourable circumstances. Percentages of 20, 12 and 10 were occasionally obtained, but were quite exceptional.

A striking fact becomes evident from Table II, namely that dilution of the serum does not arrest the process of sensibilisation of red blood-cells. When the red cells of one part of guinea-pig blood are mixed with one part (or more) of serum, sensibilisation occurs and phagocytosis results equally whether the serum is undiluted or is mixed with as much as 500 parts of 0·85-per-cent. sodium chloride solution.

The ratio noted above $\left(\frac{B}{S} = \frac{1}{1}\right)$ as representing the minimum relative amount of serum necessary for the production of marked phagocytosis enables an idea to be formed of the delicacy of the test of deopsination employed in Table I. If the red blood-cells of 0·005 c.c. of the blood of the guinea-pig are mixed with 1 c.c. of deopsinated serum, and are found to become so far sensibilised that marked phagocytosis is obtained, it follows that the serum contains, after deopsination, at least 0·5 per cent. of the amount of opsonin originally present. If, however, phagocytosis is found, on repeated testing, to be always feeble or to be absent, it follows that less than this percentage is present. By adding the red blood-cells of varying amounts of guinea-pig's blood to 1 c.c. of the serum, and noting the point at which sensibilisation becomes incomplete and phagocytosis feeble or absent—and, as is illustrated in Table II, this point is indicated with a fair amount of clearness if a sufficient number of experiments are made, the same technique being closely followed in each—it appeared to be feasible to ascertain approximately the degree to which opsonin had been removed from the serum in question.

Influenced by this line of thought, the series of determinations in Table III, which forms a continuation of Experiments 8 to 21 in Table I,

Table II.—Experiments to Determine the Degree to which Red Blood-cells must be
Rabbit A, and the Red Blood-cells

No. of experiment.	Duration of action of red blood-cells on serum.	At temperature of—	Phagocytosis* (the dilution						
			$\frac{B^*}{S} = \frac{32}{1}$.	$\frac{16}{1}$.	$\frac{8}{1}$.	$\frac{4}{1}$.	$\frac{3}{1}$.	$\frac{2}{1}$.	$\frac{1 \cdot 5}{1}$.
1	2 hours.	37° C.							— (1 in 256)
2	"	"							
3	"	"							
4	"	"							
5	"	"							+ (1 in 512)
6	"	"							
6	"	"							
6	"	"							
6	"	"							
7	"	"	— (1 in 14)	— (1 in 7)	— (1 in 7)		+ + (1 in 7)		
8	"	"			— (1 in 10)	— (1 in 10)		+ (1 in 7)	
9	"	"				— (1 in 10)		— (1 in 10)	
10	"	"				— (1 in 100)		+ + (1 in 100)	
11	"	"							

* Phagocytosis was tested by sensibilising the washed red blood-cells of guinea-pig's blood (B) with the serum

the rabbit's serum were taken. After sensibilisation the red cells were washed in 85-per-cent. sodium
0·2 to 0·6 per cent. of the leucocytes ingested red blood-cells; + + that 0·8 to 1·3 per cent. contained
instance 9 per cent., in another 10 per cent.).

was carried out. Portions of the serum of Rabbit A, diluted with an equal volume of 0·85-per-cent. sodium chloride solution, were mixed with the washed red cells of guinea-pig blood in the proportion of 4:1, for periods of time varying from 5 minutes to 3 hours and 20 minutes, and subsequently tested for the presence of opsonin, as in previous experiments (A to D, Table III). A second (E to H), and third (I to M) series of the same character were also carried out, the serum being diluted to 1 in 8 and 1 in 7 respectively. The tests of phagocytosis were made in groups, sometimes with diminishing amounts of the same serum (arranged horizontally in Table III), as in Experiments 1 to 18, sometimes with serum deopsinated for different periods of time (these experiments are marked with an asterisk

sensibilised in order to excite Phagocytosis. The Serum employed was that of
were those of the Guinea-pig.

of the serum is given in brackets).

$\frac{1}{1}$.	$\frac{1}{1\cdot5}$.	$\frac{1}{2}$.	$\frac{1}{3}$.	$\frac{1}{4}$.	$\frac{1}{6}$.	$\frac{1}{8}$.	$\frac{1}{12}$.	$\frac{1}{24}$.
	+ + + (1 in 128) + (1 in 512)		+ + + (1 in 64) + (1 in 256)		+ + + (1 in 32) + (1 in 128)		+ + + (1 in 16) + (1 in 64) + + + (1 in 16)	+ + + (1 in 8) + + (1 in 32) + (1 in 16)
	+ + (1 in 128) + + + (1 in 256)		+ + + (1 in 64) + + + (1 in 128)		+ + + (1 in 32) + + + (1 in 64)			
+ + + (1 in 8) + + + (1 in 16) + + + (1 in 32) + + + (1 in 512)								
+ + + (1 in 5) + (1 in 10) + + + (1 in 100)		+ (1 in 10) + + + (1 in 100)		+ (1 in 10) + + (1 in 100)		+ (1 in 10) + + + (1 in 100) + + + (1 in 100)		

of Rabbit A in the proportions given. Thus, $\frac{B}{S} = \frac{32}{1}$ means that 32 parts of guinea-pig's blood and 1 part of
chloride solution and added to the leucocytes. The sign — means that no phagocytosis was observed ; + that
red cells ; and + + + that the maximum degree of phagocytosis was reached (2 to 6 per cent., in one

and are arranged vertically in Table III) as in Experiments 19 to 30. In spite of the variations exhibited by individual experiments fairly definite limiting values are obtainable for most of the sera. Thus in the experiments with Serum A a sharp diminution of sensibilising power is seen when the ratio B/S is less than 1/25, whence it is concluded that 25 parts of serum after deopsination contain only as much opsonin as one part originally did, that is to say, the opsonin has been reduced to 4 per cent. of the amount present at the beginning of the experiment; with B and C a distinct but less marked change in the sensibilising power of the sera is seen, indicating reduction of opsonin to 2 per cent. and 1 per cent. respectively of the amount originally present; the opsonin in D is obviously reduced to less than

Table III.—Exhibiting the Degree of Deopsination of the Serum of Rabbit A, effected by the Red Blood-cells of the Guinea-pig acting for varying Periods of Time. Deopsination was effected by adding the washed Red Blood-cells of one part of Guinea-pig Blood to four parts of the Rabbit's Serum.

No. of experiment.	Serum.	Dilution of serum.	Time allowed for deopsination.	Temperature at which deopsination was effected.	Phagocytosis of red blood-cells of the guinea-pig, sensibilised by the deopsinated serum of Rabbit A. In carrying out these tests, the washed red blood-cells of one part of guinea-pig's blood were added to the deopsinated serum in the following amounts :—																						Approximate amount of opsonin still remaining, the amount originally present being taken at 100.
					$\frac{B}{S} = \frac{1}{500}$	$\frac{1}{200}$.	$\frac{1}{100}$.			$\frac{1}{66}$.		$\frac{1}{50}$.			$\frac{1}{32}$.	$\frac{1}{25}$.			$\frac{1}{16}$.	$\frac{1}{12}$.	$\frac{1}{8}$.	$\frac{1}{6}$.	$\frac{1}{4}$.	$\frac{1}{3}$.	$\frac{1}{2}$.	$\frac{1}{1}$.	
			h. m.		p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	per cent.
1	A	1 in 2	0 5	37° C.	4*			2*	20*						7				0		0		0		0	0	
2	,,	,,	,,	,,								12				10				0·3		0·2					4
	B	,,	0 20	,,	0*	0*	1*	3*	1*																		
3	,,	,,	,,	,,								2				0·5				0·5		0					2
	C	,,	1 20	,,	0*	1*	1·5*	2*	0*																		
4	,,	,,	,,	,,				2		0		0															1
5	D	,,	3 20	,,	0*	1*	0·4*	0·5*	0*	0					0				0		0		0		0	0	
6	,,	,,	,,	,,												0·5											< 1
7	E	1 in 8	0 5	,,						10	2*				6	10*	2*										
8	,,	,,	,,	,,																6		10		0			15
9	F	,,	0 20	,,						1·5	2*			0*	1	5*	1·5*	2*									
10	,,	,,	,,	,,																		0·5		0			4
	G	,,	1 20	,,							4*			0*		0*	1*	1*									
11	,,	,,	,,	,,																0·7		0					1·5
	H	,,	3 20	,,							2*			0*		0*	0·7*	0*									< 1·5
12	I	1 in 7	0 5	,,						5		10*	0*		8												
13	,,	,,	,,	,,																	4		0·7		0	0	12
14	J	,,	0 10	,,						6		6*	0*		1												
15	,,	,,	,,	,,																	1·4		0		0	0	> 2
16	K	,,	0 20	,,						2		5*	+*		3												> 3
17	L	,,	0 40	,,								4*	+*		0	0			0		0		0		0	0	2
18	M	,,	4 0	34°·5 C.								0*	0*		0	0			0		0		0		0	0	
		No of experiment......			19	20	21	22	23		24	25	26	27		28	29	30									

The low percentages have not the same value as the high percentages, since any imperfection of technique, in particular such as would lead to lowering of vitality of the leucocytes, or would result in defective admixture of the cells, causes diminution of the percentage noted. Hence when experiments are repeated with the same serum, the higher percentages are the more accurate. The low percentages often obtained when the amount of serum employed is relatively high $\left(\frac{B}{S} = \frac{1}{100} \text{ to } \frac{1}{500}\right)$ are due to the occurrence of agglutination, which in such cases causes difficulty, especially if the red cells have been too strongly centrifugalised.

1 per cent. of its original value, but beyond this its titre cannot be further defined from the experiments made; and similarly with the remaining sera.

The experiments in Table III show that a very rapid removal of opsonin takes place during the first five minutes, and that subsequently the removal of opsonin becomes considerably slower, some of the opsonin originally present still remaining in Sera D and H even at the end of several hours. As would be expected, the removal rate is seen to be more rapid with the more concentrated serum (A to D) than in the more dilute serum (E to H, and I to M).

The experiments in Table III show that, when spontaneous phagocytosis is avoided, the estimation of red blood-cell opsonin may be carried out. Sera from different sources may be compared as to their opsonic content by determining the minimum amount of serum required to sensibilise a given bulk of red cells so as to produce the maximum degree of phagocytosis. An alternative method is to determine the minimal bulk of red blood-cells required to deopsinate unit of volume of the sera.

The Interaction of Red Blood-cell and Opsonin.

It is not possible from the data above given to arrive at a definite conclusion as to the nature of the process occurring when red blood-cells are sensibilised by opsonin.

It has been already pointed out that even considerable dilution of the serum of Rabbit A did not abolish sensibilisation so long as the ratio $\frac{B}{S} = 1$ was preserved. This circumstance is readily explicable on the assumption that a chemical reaction takes place, but it is also equally well explicable on the assumption that a physical process of adsorption or selective solubility occurs. The relation between concentration and adsorption has been determined by Ostwald* to be

$$C_1 = aC_2^n,$$

where C_1 is the concentration of the adsorbed substance, C_2 that of the substance in solution, and a and n are constants. By suitably choosing the values of a and n, the value of C_1 could be left practically unchanged when C_2 was reduced to 1/500 of its initial value.† So far as the behaviour of opsonins in respect of dilution has up to the present been investigated,

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* 'Lehrbuch d. allg. Chemie,' 1891, vol. 1, 2te Auflage, p. 1096.

† For example, if $a = 1{,}000{,}000$ and $n = 1$, in which case (since $\frac{C_1}{C_2} = \frac{1000000}{1}$) all but 1×10^{-6} of the opsonin originally present in the liquid would pass to the red blood-cells. If n were less than 1, the proportion taken up by the red cells would be still greater. Unfortunately the estimation of very small percentages of opsonin requires much larger amounts of serum than can be obtained from rabbits.

Table IV.—The Character of the Interaction of Erythrocytal Opsonins with red Blood-cells. Temperature of Experiment 37° C. (Compare Table III.)

Experiments.	Dilution of serum.	Value of—				Relative value of—				Period.	Values of k, calculated from the formulæ—			
		t_0.	t_1.	t_2.	t_3.	C_0.	C_1.	C_2.	C_3.		$\frac{1}{t''-t'}\log\frac{C'}{C''}$.	$\frac{1}{t''-t'}\left(\frac{1}{C''}-\frac{1}{C'}\right)$.		$\frac{1}{t''-t'}\left(\frac{1}{[C'']^2}-\frac{1}{[C']^2}\right)$.
A to D, Table III	1 in 2	0′	5′	20′	80′	100	4	2	1	t_0 to t_1	0·0219	0·0025	mean 0·0016	0·0017
										t_0 to t_2	0·0085	0·0025		0·0125
										t_0 to t_3	0·0025	0·0012		0·0063
										t_1 to t_2	0·0020	0·0017		0·0125
										t_1 to t_3	0·0012	0·0012		0·0062
										t_2 to t_3	0·0005	0·0008		0·0125
E to H, Table III	1 in 8	0′	5′	20′	80′	100	15	4	1·5	t_0 to t_1	0·0165	0·0011	mean 0·0010	0·0005
										t_0 to t_2	0·0699	0·0012		0·0016
										t_0 to t_3	0·0022	0·0008		0·0028
										t_0 to t_2	0·0028	0·0012		0·0020
										t_1 to t_3	0·0013	0·0008		0·0030
										t_2 to t_3	0·0007	0·0007		0·0032
I to M, Table III	1 in 7	0′	5′	40′	—	100	12	2	—	t_0 to t_1	0·0186	0·0025	mean 0·0016	0·0069
										t_0 to t_2	0·0425	0·0012		0·0033
										t_1 to t_2	0·0020	0·0012		0·0035

the results obtained do not permit a conception of the nature of the interaction of red cell and opsonin to be formed.

In a stoichiometric reaction which proceeds slowly, not only is it possible to establish the molecular character of the reaction by means of determinations of the reaction rate, but the degree of the reaction may also be ascertained. The general formulæ for stoichiometrical reactions are

$$t''-t' = k \log \mathrm{C}'/\mathrm{C}''$$

for monomolecular reactions, and

$$t''-t' = k\,[1/(\mathrm{C}'')^n - 1/(\mathrm{C}')^n]$$

for multimolecular reactions, where C' is the concentration at time t', C'' that at time t'', n is unity for bimolecular reactions, and k is a constant. The truth of these formulæ has been established for homogeneous systems. Whether they are applicable to heterogeneous systems as in the interaction of toxin and antitoxin has been called in question. In the case of the heterogeneous system formed by red blood-cell + opsonin, the data above given enable the applicability of the formulæ in question to be tested empirically. In Table IV, from the data furnished by Table III which are repeated, the values of k are calculated: (1) as for a monomolecular reaction; (2) for $n = 1$; and (3) for $n = 2$.* The values of k for the first and third formulæ exhibit wide variations; the values for $n = 1$ show a fair correspondence, the average of all the experiments being 0·0014, while the highest and lowest values are 0·0008 and 0·0025 respectively. Thus the striking result is obtained that in the heterogeneous system under consideration a relation is exhibited similar to that observed in a bimolecular stoichiometric reaction occurring in a homogeneous system.

Summary.

It has been shown that—

1. By employing phagocytosis as a test of the presence of red blood-cell opsonin, and avoiding spontaneous phagocytosis by suitable conditions of experiment, quantitative determinations of the opsonic content of serum may be made.

2. In the experiments made, the interaction of opsonin and red blood-cell proceeded at a rate corresponding to that exhibited in a bimolecular stoichiometric reaction.

In conclusion, I must express my indebtedness to the assistance I have received from Dr. Allan Macfadyen, in whose laboratory this research was conducted, and from Mr. J. A. Craw and Professor Donnan.

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* The concentrations of red blood-cell and of opsonin are regarded as equivalent, since the opsonin is present in the minimum proportion necessary to lead to full sensibilisation of the red blood-cells.

The Structure of Nerve-fibres.

By J. S. Macdonald, the University of Sheffield.

(Communicated by Professor Sherrington, F.R.S. Received July 31,—
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Frog's nerve-fibres present very different appearances in different portions of their length, when teased and examined in Ringer's solution. The main distinctions affect the contents of the myelin sheath. To avoid misunderstanding I propose to speak of this portion of the nerve-fibre as the "intramyelin material," since the term more generally used ("axis-cylinder") has now become so completely identified with new appearances produced in this material by the action of reagents.

Briefly summarised the main differences observed are as follows:—

(*a*) In places the intramyelin material exhibits the perfect transparency and homogeneity of a clear solution.

(*b*) In places this limpidity is clouded by a fine punctate granulation.

(*c*) At others the granular appearance is much more marked. The individual granules are of different sizes, and vary from obvious spherules to just perceptible points.

(*d*) In other places again large hemispherical vacuoles make their appearance, their bases upon the myelin sheath and their convex surfaces pointing inwards towards the central axis of the fibre. These marginally placed vacuoles are situated at irregular distances from one another, and are of irregular size. They lie now on this side, now on the other side of the fibre. The remainder of the intramyelin material, the more central portion, is here less translucent and more granular.

(*e*) The vacuoles here have extended, and by their more numerous formation and communication with one another have joined to form an irregularly spiral channel. The central and less translucent portion of the intramyelin material also presents the appearance of a spiral, as it bends now to this point, now to that point upon the myelin sheath at places where a separation from the myelin sheath has not yet occurred. The curves of this irregular spiral are now short, now long. The material of which it is formed also varies in diameter, more swollen here, more condensed there. The central mass is much longer than the straight tubular myelin sheath within which it lies.

(*f*) In other places this central coagulum is of more uniform diameter and forms a straighter line. It has undergone a diminution in length and in thickness.

The evidence of this change of length is sometimes very complete. Imagine a stretched elastic string placed in the long axis of a tube with flexible walls. Release the string and so let its elasticity come into play. As the string contracts, the flexible tube will become arranged as a spiral curled around the axial string. Such a figure would be the reverse of the appearance described above in which a straight tube enclosed a spiral string. I do not say that I have seen evidence of tension so complete as this, but I have frequently seen something similar, if something less.

Thus when the process of teasing has left nerve-fibres arranged upon the slide in undulating curves, the clot which forms within the intramyelin material sometimes shortens to a length less than that of the fibre. In this case the clot does not follow the curvature of the fibre, but passes straight across from one point of maximal curvature to another.

Sometimes this central coagulum may be seen to show the usual signs of longitudinal strain in a viscous cord. It is faintly marked by fine lines in the main direction of the strain, and has a finely fibrillated appearance which has led to a belief that this central mass is formed by the agglutination of a number of originally separate "neuro-fibrils." There is, however, nothing in the appearances seen in teased nerve-fibres to warrant such a view. The most severe test to which this view can be put is to seek, in terms of it, for an explanation of the alterations in length which this central mass undergoes. Such an explanation would require not only an imagination of separate fibrils, but also their new endowment with the property of elasticity.

In nerve-fibres teased in Ringer's solution these appearances are usually distributed in a definite order. The immediate vicinity of the cut end is granular, this granulation is succeeded by a tract of homogeneous intramyelin material, and then all the successive appearances due to a gradually increasing degree of coagulation occur in the order given above. The order in which the appearances are arranged is sometimes complicated by less extensive and subsidiary sets of appearances of the same kind arranged around interpolated nodes of Ranvier.

This balancing of appearances around injured points and nodes of Ranvier introduces a complexity into the description of the normal character of teased nerve-fibres. If there is any hurry to identify some one of these appearances as the "normal" one, it must be remembered that teased nerve-fibres are traversed by the "current of injury" and must be expected to exhibit evidence of consequent polarisation.

Nerve-fibres Teased in "Normal Saline" Tinged with Toluidine Blue.

I have, in a previous note, described the manner in which these nerve-fibres are affected by the addition of neutral red to the "normal saline" in which they were teased. This dye characteristically stains all injured points and some of the nodes of Ranvier. As time elapses it also stains granules which form at gradually increasing distances from these points. The most notable fact about neutral red staining is the slow invasion of the nerve-fibre by this process of granule formation. When I first replaced this basic dye by another one of an apparently not very different character—toluidine blue—I was surprised by the complete difference in result obtained. It is true that here and there some stained plugs of material were visible at injured points, but I failed to find any trace of the progressive granule formation so evident in the other case. The characteristic effects of toluidine blue staining were obtained in quite another region of the teased nerve-fibres, and had a very different character. This dye stains all those regions of the nerve-fibre in which the coagulative changes occur, whereas neutral red yields a picture of a slowly progressing change gradually affecting that first portion of the nerve-fibre in which the intramyelin material is at first seen homogeneous and transparent.

In these regions of coagulative change toluidine blue gives rise to appearances which vary *pari passu* with the extension, and with the intensity, of the coagulation present. Regions such as in unstained fibres would be definitely granular, are loaded with blue granules distributed with much precision across and along the intramyelin material. Such granules leap suddenly into sight over long stretches of the fibre, and are not originated therefore by any process which can be described as an invasion. The tract of developed granules is bounded on the side nearer to the injury by a clear, unstained and homogeneous region, the line of demarcation is sometimes remarkably sharp. On the other side the granular tract passes into a region in which coagulative changes have led to the development of a central rope now stained an intense blue colour.

This curiously complementary behaviour of these two basic dyes is therefore of obvious interest in mapping out two different tracts of the teased nerve-fibre. It certainly does not diminish this interest that in case of either dye an appropriate adjustment in the saline solution in which the dye is introduced to the nerve-fibre may be made to overcome this dissimilarity. If Ringer's solution is substituted for "normal saline," or, better still, if a small quantity of sodium carbonate, 0·02 per cent., is added to the Ringer, or even when the sodium carbonate is simply added to "normal saline," or even when the basic dye is itself added in increased quantity, then toluidine blue may be made to reveal the complete set of appearances. In these solutions the invasion of the homogeneous region may be watched in a nerve-fibre in which the sudden appearance of granule formation in more distal regions and the intensely stained coagulum is also marked. If, on the contrary, Ringer's solution is substituted for "normal saline" in the case of neutral red, nothing whatever, or at the most only faint traces of granule formation are obtained even in the region near to the cut end. The neutral red stain is enabled to penetrate the whole nerve-fibre best when present in small quantity, 0·01 per cent., and when presented in unboiled "normal saline" rendered faintly acid by the presence of carbonic acid. Toluidine blue solution must be rendered faintly alkaline, or, at least, the calcic phosphate of the Ringer's solution must be present to prevent a development of acidity. Neutral red is, on the other hand, ineffectual in the presence of a reagent, preventing the development of acidity, and can be made to stain the distal coagulated stretch of the fibre only when presented in association with a slight trace of acid. There is an obvious conclusion to be drawn from this series of facts.

There is one region of the nerve which neutral red stains when unaided by any addition of acid. This is the point of actual injury, and a limited region in its neighbourhood which gradually extends with lapse of time. The inference is clear, that this is a tract of the nerve in which a certain amount of acidity develops, or, at least, that it is the least alkaline portion of the nerve. The behaviour of toluidine blue in the same region is also seen to support this inference. Here it refuses to stain until alkalinity is obtained. This might lead to the additional inference, that the region of coagulation which toluidine blue stains unaided is itself more alkaline than any other tract of the fibre.

It would seem therefore that at present we had done no more than map out the teased nerve-fibre into regions of homogeneity and of coagulation, into regions of less and greater alkalinity, and also that we have associated these two separate attributes. There is a homogeneous region close to each injured point which is gradually invaded and rendered granular by an acid tide sweeping in from the point of injury. There is a more alkaline region, in which different grades of coagulation occur.

The Distribution of Potassium Salts in Teased Nerve-fibres.

I have already pointed out that Macallum's reagent may be used to prove the existence of potassium in every portion of the length of a nerve-fibre provided only that the portion tested be first injured previous to the application of the test. The circumstance of injury reveals the presence of a highly concentrated solution of potassium salt within the intramyelin material, which makes an immediate appearance at every injured point. The bizarre appearances produced by this method of mapping out injured points had been observed by Macallum, but was left completely unexplained by him. Their relation to injury is, however, unmistakable when sought for. Since these points of injury can be multiplied indefinitely so as to include the whole length of the nerve-fibre, it follows that potassium really exists everywhere within the intramyelin material in some manner hidden from the action of the reagent, and that the processes attendant upon injury are capable of presenting it in a new condition ready to take part in the reaction. I have discussed the probable meaning of this fact in a previous communication, and the important conclusions which can be drawn from it as to the nature of nerve-function.

A slight modification in the method of use of this reagent brings out further points of considerable interest. For if nerve-fibres are teased in "normal saline" and allowed to lie in this solution for some time before being tested with Macallum's solution, the potassium solution is found much more widely distributed. It is now found in all those regions of the fibre, in which toluidine blue can be used to reveal its own characteristic effects. Under these conditions potassium is discoverable at each site of injury, and in a neighbouring tract to which it has diffused from this site. Potassium is also now detected in all those regions in which I have described the occurrence of coagulative change. We are, therefore, introduced to a new and very important conclusion. Potassium salts are also discoverable in regions of the fibre distant from points of injury, provided that time is given for their appearance.

Although not situated at injured points this more distal region of the fibre has a secondary relation to injury of a most interesting kind. This relation is at once made evident on reference to Bethe's observations upon the staining capacity of polarised nerve-fibres.

Bethe has made use of an ingenious experimental method, whereby the "fixation" of a nerve is secured during the traverse of a polarising current. The nerve is then prepared for examination under the microscope, and is stained in toluidine blue or in some other similar basic dye. Prepared in this way the fibres are found to exhibit a remarkable distribution of staining capacity. The anodal region is pallid, the indifference point is studded with granules, and the kathodal region is intensely stained. It will be seen that this set of appearances is exactly similar to that which I have observed in teased nerve-fibres stained with the same dye. Now I have already produced evidence in favour of the view that teased nerve-fibres are traversed by an electrical current—their own injury current. Bethe's observations enable me therefore to identify this distribution of staining capacity in teased nerve-fibres as due to polarisation caused by this current. The pallid stretch of homogeneous intramyelin material is the anodal region of the self-polarised nerve. The deeply-stained region, in which coagulative changes are observable, is the kathodal region where the injury current leaves the nerve-fibre to traverse the external circuit of the salt solution. We can now return to the evidence obtained as to the distribution of potassium.

Injury suddenly elicits the appearance of strong solution of potassium at the injured point. Given time, this solution diffuses into the neighbouring stretch of the nerve-fibre. This phenomenon is accompanied by an electrical phenomenon, the injury current. The traverse of the injury current polarises the fibre and gives rise to the appearance of a new mass of potassium solution, accompanied by appearances characteristic of coagulation at the kathodal region.

Macallum has more recently published an account of the distribution of the inorganic chlorides present in nerve-fibres. With the assistance of Miss Menten he has penetrated the false character, which the vagaries of the slowly-diffusing silver nitrate confer upon their apparent distribution, and has discovered the fact that the inorganic chlorides are everywhere present in great quantity in the intramyelin material. The drawings accompanying this paper also reveal a very remarkable addition to this statement of chloride distribution. In most of these illustrations it is easy to observe indications of effects produced by the nature of the reagents made use of. The proportion existing between the diameters of the myelin sheath, the intramyelin material, and the whole fibre, are alone sufficient basis upon which to make this statement. The intramyelin material does not occupy one-half of its usual relative cross-section. Where this is not the case, and the right proportions are maintained, the authors describe the fibres as swollen. Wherever, in the illustrations, this swollen, but normal, appearance of the fibre is present, the distribution of the chloride coincides exactly with the appearance of teased nerve-fibres stained with basic dyes, and also with appearances obtained by the use of Macallum's reagent for potassium. I have no hesitation whatever in urging, upon this basis of evidence, the necessity of conjugating these facts. The salt present is potassium chloride. It is present in extraordinary quantity. It is present in some masked position in the structure of the intramyelin material. From this position there is direct evidence that it is enticed by injury, and by the kathodal state.

Staining Due to Salt Concentration.

We may now turn to another striking fact which has made its appearance in the course of these investigations. Basic dyes, toluidine blue, and neutral red have been made use of to map out the distribution of a material, which, upon further examination, turns out to be potassium chloride. Set forth in these direct terms, there is nothing surprising in this statement, since both dyes can be "salted out" from their solutions by the addition of potassium chloride. Neutral red is a most insoluble body when tested in this manner, and it was for this reason that I selected its staining effects as a guide to the distribution of the potassium chloride solutions in nerve-fibres. Toluidine blue is more soluble, nevertheless it is easily capable of being "salted out" by solutions of the concentration that my electrical experiments have led me to anticipate. I have also since found that it is more easily "salted out" by potassium chloride in the presence of small traces of alkali, 0·03 sodium carbonate. This latter point has an obvious bearing upon the salting out of toluidine blue in alkaline cell-structures, and more especially at kathodal points.

This definite coincidence between toluidine blue staining and results obtained by the use of reagents precipitating potassium and "chloride" renders it possible at once to advance a general proposition of some interest: *All the appearances made evident in the nerve-fibre by the use of toluidine blue are due to the manner in which this dye is salted out from its solutions by potassium chloride.*

There is also a further point. The appearances which Bethe developed in nerves fixed in alcohol, or in alcohol and ether, I have obtained in nerves freshly teased in "normal saline." Bethe's results coincide with mine, and must be equally accepted as providing a picture of solutions of different concentrations of potassium chloride. Nor is this to be wondered at. Potassium chloride is only slightly soluble in alcohol, although extremely soluble in water. The addition of alcohol, the rapid dehydration which ensues, and the replacement of the water by alcohol, must be definitely expected to leave the salts present as precipitates. Wherever it exists the potassium chloride is precipitated practically in the same quantity as it was originally present in solution. Alcohol fixation, therefore, is precisely calculated to leave *in situ* precipitates of salt for subsequent micro-chemical investigation with toluidine blue and similar dyes.

It is impossible to leave this point without remarking upon the general bearing of this conclusion. The nerve-fibre cannot be the only case in which considerations of this kind are worthy of examination. Granules in the cell-bodies of secretory cells, or in the bodies of nerve-cells, are no doubt elicitable by similar means, which also have the same significance. It is possible that Nissl granules are of this order of importance. Failing definite evidence upon which to make any assertion, it must at least be said that the well-known appearances accompanying "chromatolysis" are extremely suggestive in character. They would seem to be the consequences of such changes in osmotic pressure as would result from the sudden liberation of salts into solution.

Neurofibrils.

It is now possible to deal with the phenomena observed in that tract of the teased nerve-fibres which corresponds with Bethe's indifference point and its granules. In teased nerve-fibres this region is naturally much more extended, since the polarising "injury current" has a comparatively small value and the changes in the intrapolar region are more gradual. Throughout the whole of this region dense granule formations occur, and these are of such a striking appearance when examined with high powers of the microscope as to absorb a major share of attention. In my own case their fascination left me at first with scant respect for the polar changes described above. To a certain extent this interest is justified when it is considered as the region least involved in polar changes, and altered, therefore, only from the "normal" resting condition in so far as it is involved in the transmission of an electrical current. For the present, however, it is sufficient to deal with the light which these granule formations throw upon the artificial structures known as neurofibrils.

In a previous communication I reported the fact that in nerve-fibres teased in saline solution I had never seen structures bearing any very close resemblance to the neurofibrils described as making their appearance in nerve-fibres "fixed" and stained in special reagents. Since making this statement I have, however, seen structures which cannot be described as granules, since their length is considerable and their thickness much too minute, and which yet cannot be called "neurofibrils," since they are too short, too irregularly arranged, and since they anastomose. The resemblance which they bear to the "neurofibrils" described as found in fixed nerve-fibres is very much that borne by the anastomosing networks of fibrils described as structural components of some nerve-cells. Fibrils such as this I have only seen in some few out of the many nerves that I have teased. Much more frequent, but still only of comparatively occasional occurrence, are structures conveniently classed as bacillar granules, shorter but more characteristically much thicker than the fibrils. The latter are always in much smaller number in each unit length of the fibre. Other forms seen far more frequently are short ovoid and spherical granules and flakes of the precipitated dye. When all these forms are present they occur in a definite order. The fibrils are found in the first portion of this stained region abutting upon the unstained anodal tract, then follow bacillar granules, then ovoid granules always associated with tracts of the fibre in which definite kathodal staining and coagulative changes are found. In this latter region spherical granules may also occur, but they are placed not in the central coagulum but in the clearer fluid surrounding it. In one and the same portion of the kathodal region ovoid granules may be seen enclosed in the central mass, flakes may be seen upon its surface, and spherical granules may be seen in the clear fluid surrounding it.

To understand the meaning of these granule formations and this difference in their character, it is essential to remember the manner in which they are thus associated with different degrees of coagulation. At the distal extremity of the homogeneous anodal tract is placed the region in which the first approach to visible coagulation takes place. In this place there is no separation between a core and a surrounding fluid. Each unit of volume still contains almost its normal amount of uncoagulated material. The threads of coagulum are still surrounded by an almost normal intra-myelin solution. It is in this region, where " solid phase " is still surrounded by a great mass of " fluid phase," that the fibrils are sometimes seen. Before I had puzzled out the meaning of the adjacent unstained anodal tract, in which no granule formation takes place except as the result of diffusion from the source of the injury current, I was greatly exercised by this constant position of such fibrils as I had found. The bacillar granules probably represent a further stage of degradation, in which, however, the main mass of material is almost normally fluid in character. In both these cases the dye is precipitated upon the outer surface of threads of coagulum.

As we proceed into districts nearer and nearer to the kathode an opposite set of conditions arises. The greater portion of the proteid material is precipitated or coagulated as a " solid phase." Within the interstices of this mass there are, however, entrapped collections of solution. It is probable that here the granules represent such collections within which the dye is precipitated.

At one end of the scale of changes solid threads are stained upon their surfaces. At the other there is a similar staining upon the surface of a coagulated mass, and within this again are intensely stained vacuoles.

In no place are neurofibrils of indefinite length existent, simply by reason of the gradually varied conditions present in the district where coagulation is taking place.

REFERENCES.

A. B. Macallum, "On the Distribution of Potassium Salts in Animal and Vegetable Cells," 'Journal of Physiology,' vol. 32, pp. 96–128, 1905.

A. B. Macallum and Miss Menten, "On the Distribution of Chlorides in Nerve Cells and Fibres," 'Roy. Soc. Proc.,' B, vol. 77, pp. 165–192, 1906.

Albrecht Bethe, "Allgemeine Anatomie und Physiologie des Nervensystems," pp. 272–298, Leipzig, 1903.

J. S. Macdonald, "The Structure and Function of Nerve Fibres," 'Roy. Soc. Proc.,' B, vol. 76, 1905.

The Influence of Increased Barometric Pressure on Man.—II.

By Leonard Hill, F.R.S., and M. Greenwood, Jr., M.R.C.S., British Medical Association Research Scholar.

(Received October 6,—Read November 8, 1906.)

In a previous communication, we gave reasons for thinking that decompression symptoms may be avoided by maintaining a steady rate of 20 minutes per atmosphere during this process.

The actual time selected was purely empirical, being based on the statistics of Caisson Works, Diving Operations, and Laboratory Experiments. It is clear that a more scientific foundation would be obtained if we could determine (1) the rate at which the tissue fluids are saturated with nitrogen; (2) the rapidity with which dissolved gas escapes during decompression. The most direct method would be to analyse samples of arterial blood under various pressure conditions; but this is not, unfortunately, practicable in the case of man. Another way is the examination of venous samples under similar circumstances. This plan can be followed, and we hope to communicate some results in another paper, but the technic is difficult and still in need of improvement. A third line of research is the indirect determination of the tissue gases, and this will be discussed in the present communication.

If a condition of diuresis be produced by drinking considerable amounts of water, the profuse secretion of urine which results will afford some measure of the dissolved tissue fluid gases. We have proceeded in the following way:—The subject of the experiment drinks at least a quart of warm water, and, after an interval of 10 to 15 minutes, enters the pressure chamber, the pressure being then raised a definite amount. Directly the desired pressure has been attained, he empties his bladder. Ten minutes later the bladder is emptied again. Samples of the urine passed are then run into glass bulbs with rubber connections, care being taken to drive out any air bubbles that may be present. The rubber junctions are then clamped, and the dissolved gas subsequently pumped out by means of Hill's blood-pump, the volume determined, and the mixture analysed by the potash and pyrogallic acid method.

In this manner, a sample of urine, passed since the pressure limit had been reached, was available for analysis, and a series of specimens could be obtained. It was first, of course, necessary to determine the amount of gas dissolved by the urine under normal pressure conditions. Six analyses were made, and the mean volume of dissolved nitrogen per 100 c.c., reduced to 760 mm. and 0° C., was found to be 1·14 c.c. The individual observations, made at different times and under different circumstances, exhibit only a small range of variation, they are 1·21, 1·19, 0·97, 1·10, 1·31, 1·07, 1·14. The coefficient of variability determined by one of us (M. G.) is only $9{\cdot}5699 \pm 1{\cdot}8799$.

We are thus entitled to assume that, in our own cases, the coefficient of solubility for atmospheric nitrogen under normal barometric pressure, and at body temperature, is between 0·11 and 0·12.

We next determined the changes in the amount of dissolved nitrogen with varying pressures. The following are the results of a typical experiment:—

Experiment 31, 31.5.06.

M. G.—compressed to +30 lbs. in 10 minutes (2.50 p.m.).

The bladder was then emptied, and at 2.55 the bladder was emptied again, 135 c.c. of urine being passed, and Sample 1 was taken.

2.56—decompression commenced.

3.20 (+15 lbs.)—the bladder was emptied again (320 c.c.).

3.45—decompression completed, bladder emptied again (310 c.c.).

4.1—another sample taken (135 c.c. passed).

At 4.55 and 6.10 other samples were taken.

Analyses.

Sample.	Time and pressure.	Percentage of nitrogen.	Calculated N per cent., supposing urine followed the pressure.
1	Passed after 5 minutes under 30 lbs.	2·522	1·1 × 3 = 3·3
2 and 3	Were spoiled		
4	At normal pressure, 16 minutes after decompression	2·373	1·1
5	70 minutes after decompression	1·107	1·1
6	245 minutes later	1·023	1·1

In another experiment the following were the results :—

Sample.	Time and pressure.	Percentage of nitrogen.	Calculated N per cent., supposing urine followed the pressure.
1	45 lbs., immediately	2·64	1·1 × 4 = 4·4
2	„ 8 minutes later	4·57	1·1 × 4 = 4·4
3	„ 9 „	4·69	1·1 × 4 = 4·4
4	„ 13 „	4·35	1·1 × 4 = 4·4
5	„ 23 „	4·57	1·1 × 4 = 4·4
6	At 30 lbs. during decompression	4·252	1·1 × 3 = 3·3

These results, amply confirmed by our other experiments (*vide* tables, pp. 26 and 27), merit some consideration.

First of all, we note that an exposure of more than five minutes to a positive pressure of 30 lbs. is necessary in order to produce saturation; the mean of five samples taken under these conditions is 1·99, as compared with 3·3, the theoretical value.

Saturation appears to be attained in 10 minutes,* however. Thus, the mean of seven samples, taken not less than 10 minutes after attaining the above-mentioned pressure, was 3·32. For a pressure of +45 lbs. the same statement appears to hold, but the time limit is probably slightly lower, as, on two occasions, full saturation was obtained in eight minutes. The mean of eight observations at this pressure, for exposures of less than 10 minutes, is 4·23, the theoretical value being 4·4.

The next point to notice is that the escape of dissolved gas is a much slower matter than could be expected. Thus, in experiments at +3 atmospheres, samples were taken when the pressure had fallen during slow decompression to +2 and +1 atmospheres. The means of 7 and 8 observations at these pressures were, respectively, 3·63 and 2·75, *i.e.*, the samples were super-saturated.

These results, however, are not surprising, since some of the urine taken in the specimen must have been separated from the blood while under the higher pressure, hence we could not assert, on the strength of these observations alone, that any definite retardation occurs in the equilibrating process. But, if we consider the analyses made after returning to the normal pressure, we find very strong evidence in support of such a view. In the experiment recorded above, a sample passed *sixteen* minutes after decompression contained 2·373 per cent. of nitrogen, in another experiment the amount was 1·66, the mean of 5 being 1·64.

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* Saturation of the kidney under the conditions of diuresis is probably far quicker than that of other and less vascular organs.

Of samples passed immediately after decompression not one yielded less than 1·64 per cent., the mean of 6 being 1·99.

We are thus led to conclude that, even with a decompression rate of 20 minutes per atmosphere, the excess of dissolved nitrogen has not escaped entirely from the tissue fluids, even 15 minutes after decompression. As the matter is of importance, we must examine the validity of our method a little more closely. The accuracy of the analytical process itself was tested by submitting a sample of boiled water to a vacuum in the Hill pump and proceeding exactly as in the urine analyses. No measurable gas was obtained, so that the error due to leakage may be regarded as negligeable. In any case, we disregard analyses in which, owing to the presence of unusually large quantities of oxygen, we can reasonably suspect contamination with air. In general, only very small quantities of oxygen were found.

The admixture of urine that had collected in the bladder while the subject was exposed to a higher pressure would lead to erroneous results, but the *complete* emptying of the bladder at each observation avoids this source of fallacy. All the urine of the samples passed 15 minutes after decompression must have been separated from the blood after the pressure had fallen to + 0 lb., with the exception of the amount present in the tubules and renal pelves, this latter quantity being so small in comparison with the total volume (135 c.c.) passed, that the resultant error can hardly be appreciable.

That our experiments are not sufficiently numerous to admit of definite statement as to the exact time relations of the process may be granted; but the qualitative accuracy of the conclusion seems unassailable.

We have obtained a striking confirmation of our view in the following manner. At the caisson works now being carried out by the London County Council in connection with the new Rotherhithe Tunnel, a positive pressure of 14—17 lbs. is employed and only three minutes allowed for decompression.* We have analysed samples obtained from six caisson workers obtained directly after leaving the air lock, with the following result:—

	Nitrogen.
1†	1·595
2	1·658
3	1·506
4	1·414
5	1·747
6	1·744

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* With such low pressures there is little risk in so short a decompression period.

† The men had some beer to drink and most of them passed water just before decompression.

It must be admitted that these observations indicate in a manner not to be disregarded the extreme danger of rapid decompression from high pressures. It might even be doubted whether 20 minutes per atmosphere is an absolutely safe allowance; indeed, a few fatal cases, recorded in the literature of caisson works, in which this time appears to have been allowed, support such a contention. It may well be, however, that a certain steepness of pressure-slope between dissolved and free gas is necessary for the formation of bubbles sufficiently large to produce injury.

So far as our observations go, with a decompression rate of 20 minutes an atmosphere the dissolved nitrogen falls in a given time to the same level, whatever be the height of pressure attained during the experiment. That is to say, the excess of dissolved nitrogen on returning to zero is no greater after exposure to three atmospheres than after raising the pressure to two atmospheres only. It would seem that the velocity of escape diminishes from some critical point. If this be so, it would be an argument for retarding the later stages of decompression in order that an equilibrium may be re-established on returning to normal conditions.

The causes underlying these results remain for subsequent consideration; in the light of other experiments we have carried out, we think it possible that a physical explanation will be found adequate to account for the facts, but our investigations are not yet sufficiently complete to permit its immediate statement.

A complete table of our analytical results is subjoined; the figures queried are possibly vitiated by experimental errors, we have no reason to doubt the accuracy of the others.

Our general conclusions from the present investigation are :—

(1) The body fluids of man exposed to compressed air absorb nitrogen in accordance with Dalton's law.

(2) Saturation of the body fluids is attained after exposure to pressures of +30—45 lbs. for from 10 to 15 minutes.*

(3) Even with a decompression rate of 20 minutes an atmosphere, equilibrium between dissolved and atmospheric nitrogen is not completed 15 minutes after decompression.

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* Our results agree in principle with those deduced by V. Schrötter from theoretical considerations, *vide* 'Sauerstoff in der Prophylaxie und Therapie der Luftdruckerkrankungen,' Berlin, 1904, pp. 35—39.

Table I.—Urine Analyses from Hill and Greenwood's Experiments. Individual Observations.

Pressure.	N_2 per cent. found.	Means.	N_2 per cent. calculated, supposing urine followed the pressure.
+0 lb.	1·21, 1·19, 0·97, 1·10, 1·31, 1·07..............	1·14	1·1
+30 lbs. for less than 10 mins.	2·52, 1·67, 1·83, 2·41, 1·5	1·99	3·3
+30 lbs. for more than 10 mins.	3·32, 3·49, 3·29, 3·22, 3·42, 3·94, 2·57	3·32	3·3
+45 lbs. for less than 10 mins.	2·64, 4·57 (8 mins.), 5·68 (?) (8 mins.), 4·28	4·29	4·4
+45 lbs. for more than 10 mins.	4·54, 3·78, 4·52 (?), 4·69, 4·35, 4·57, 3·46, 3·94	4·23	4·4
+30 lbs. during decompression	4·01, 3·47, 3·52, 4·25, 3·68, 3·13, 3·33	3·63	3·3
+15 lbs. during decompression	2·40, 2·78, 2·96, 3·16, 2·60, 3·04, 2·35, 2·73	2·75	2·2
+0 lb. on decompression	1·84 (4 mins.), 2·21, 1·70, 2·30, 1·64, 2·24	1·99	1·1
+0 lb. 15 mins. after decompression	1·34, 1·37, 2·37, 1·66, 1·45 (10 mins.)	1·64	1·1
+0 lb. more than 15 mins. after decompression	1·28, 1·07, 1·02	1·13	1·1

Table II.

A. Rotherhithe Tunnel Workers.

Source of sample.	N_2 per cent. found.	N_2 per cent. calculated, supposing urine followed the pressure.
In tunnel (+17 lbs.)	2·38, 1·83	2·35
After decompression (decompression time, 3½ mins.)	1·60, 1·66, 1·51, 1·41, 1·75, 1·74, 1·31, 1·63	1·1

B.* From Admiralty Diver C.

Depth.	Time below and N_2 per cent. found.	N_2 per cent. calculated, supposing urine followed the pressure.
80 feet	Below 46 mins. 20 secs. Ascended in 6 mins. 5 secs.—Sample taken 20 mins. 30 secs. after ascent: N_2 = 1·73	1·1
84 "	Below 46 mins. Ascended in 5 mins.—Sample taken 11 mins. after ascent: N_2 = 1·60	1·1
72 "	Below 44 mins. Ascended in 6 mins.—Sample taken 5 mins. after ascent: N_2 = 2·11	1·1
	" 20 " " N_2 = 1·03	1·1
80 "	Below 46 mins. Ascended in 4½ mins.—Sample taken 4 mins. after ascent: N_2 = 2·50	1·1
	" 20½ " " N_2 = 1·38	1·1

* For these samples we are indebted to Dr. Haldane, Lieutenant Damant, and Mr. Cato.

The experiments were carried out with the help of a Government Royal Society Grant, and with the aid of apparatus placed at our use by Messrs. Siebe, Gorman and Co.

On the Inheritance of Certain Invisible Characters in Peas.

By R. H. Lock, M.A., Fellow of Gonville and Caius College, Cambridge.

(Communicated by W. Bateson, F.R.S. Received October 1,—Read November 22, 1906.)

In a paper which appeared recently in the 'Proceedings of the Royal Society,' Messrs. Bateson and Punnett and Miss Saunders showed that the visible appearance of certain characters in Sweet Peas and in Stocks was due to the association together in the same zygote of factors belonging to independent but complementary allelomorphic pairs. If both the complementary factors were not simultaneously present, no hint of the presence of the characters in question was to be detected without breeding tests; nevertheless, the presence and absence respectively of such a factor constitute a pair of allelomorphs, and segregate in complete accordance with Mendel's law.

The present communication deals with a similar but somewhat simpler phenomenon exhibited by certain colour characters of the testa of the ordinary field or garden pea—the species to different cultivated varieties of which the names *Pisum sativum* and *P. arvense* have been given. The case is simplified by the fact that here only one of the two complementary factors is by itself invisible. The presence of the second factor is invariably accompanied by the appearance of a definite visible character, whether the invisible factor is present or not. The present examples of "latency" are of special interest on account of the classical nature of the material, as well as because it has been suggested that Mendel was in error in his own account of the characters of the testa in peas. It may be stated at once that this is not the case. Mendel's account is perfectly correct so far as it goes. The grey colour of the testa, constantly associated with coloured flowers and coloured axils, is a simple Mendelian dominant to colourless testas coupled with white flowers and green axils.

The testa may, however, show other colours in addition to the well-known grey. In Ceylon, in 1902, the writer found in cultivation a pea, the appearance of which strongly suggested that it came very near to the primitive ancestor of cultivated peas. We are concerned here only with the characters of the testa. This showed an essentially grey ground colour (greenish or yellowish), and, in addition, two distinct sorts of coloured markings:—(1) small specks of a very deep purple colour, the presence of which may be briefly denoted by the letter *p.*; and (2) a mottling or marbling of brown, very distinct, and closely resembling the pigmentation which is characteristic of the cultivated variety known as "maple"; the presence of this character will hereafter be referred to as *m.* The grey ground colour may further be denoted as *g.* In the absence of these three characters the testa of a pea is commonly nearly colourless, and this absence of colour in the testa is constantly associated with white flowers and unpigmented axils. The latter group of characters we shall call *w.* The native pea with which the following experiments were made bears, then, the characters *m.p.g.* Its flowers were purple and its axils coloured.

At a recent meeting of the Royal Horticultural Society, Messrs. Sutton exhibited a cross between a white seeded variety and what was described as a possible wild type of *P. sativum*, seeds of which had been obtained from a plant found in Palestine by Mr. Arthur Sutton. On examining the seeds of this cross, I found, as I had expected, that they showed both the purple spots and the maple marking on a grey ground colour.

Of the colour characters referred to, the grey is determined by a greenish insoluble pigment contained in all, or almost all, the cells of the testa. The purple pigment exists in the form of a coloured cell sap, confined to certain large cells of the subepidermal layer. The brown pigment seems to exist chiefly in the cell walls of the outermost layer of I-shaped cells.

Early in 1903, the Ceylon variety, already described, was crossed with an English pea known as "Sutton's Satisfaction," having colourless testas. The testas of the seeds borne by the resulting plants were all precisely like those of the native parent—*m.*, *p.*, and *g.* are all dominant.

In F_2 only 25 plants were raised, which showed the following characters:—

m.p.g.	*m.g.*	*p.g.*	*g.*	*w.*
11	2	6	2	4

In the case of one of the four plants with white flowers, the nearly colourless testas exhibited a very faint brown marbling, like the "ghost" of a maple character.

These few plants were clearly insufficient to allow of any deduction being made as to the characteristic proportion of F_2. Three of the above *m.p.g.* plants, however, yielded once more all the different types in F_3, being, therefore, clearly of the same heterozygous constitution as their parents; 169 plants of this description included the following:—

m.p.g.	*m.g.*	*p.g.*	*g.*	*w.*
82	20	21	9	37

And the offspring of the remaining plants showed nothing to contradict the following theoretical explanation, which was published as a provisional hypothesis in 1904.*

I regarded *m.*, *p.*, and *g.* as each depending on the presence of the dominant member of a separate pair of allelomorphs A—a, B—b, and C—c respectively, C representing the grey character. And I further supposed that A and B, though present, were unable to lead to the appearance of *m.* and *p.* in the absence of C. B (*p.*) was totally inhibited in this way, whilst A might just make its presence known by the appearance which we have described as that of "ghost" maple.†

In the accompanying diagram the combinations of the three pairs of allelomorphs are set down at length, and it will be seen that, on the above hypothesis, they lead to the proportion—

m.p.g.	*m.g.*	*p.g.*	*g.*	*w.*
27	9	9	3	16

with which the result actually obtained and given above is in very fair agreement. The expectation is 71.3, 23.8, 23.8, 7.9, 42.2, and the observed numbers were 82, 20, 21, 9, 37.

	ABC	AbC	aBC	abC	ABc	Abc	aBc	abc
ABC	ABC ABC	ABC AbC	ABC aBC	ABC abC	ABC ABc	ABC Abc	ABC aBc	ABC abc
AbC	AbC ABC	AbC AbC	AbC aBC	AbC abC	AbC ABc	AbC Abc	AbC aBc	AbC abc
aBC	aBC ABC	aBC AbC	aBC aBC	aBC abC	aBC ABc	aBC Abc	aBC aBc	aBC abc
abC	abC ABC	abC AbC	abC aBC	abC abC	abC ABc	abC Abc	abC aBc	abC abc
ABc	ABc ABC	ABc AbC	ABc aBC	ABc abC	ABc ABc	ABc Abc	ABc aBc	ABc abc
aBc	aBc ABC	aBc AbC	aBc aBC	aBc abC	aBc ABc	aBc Abc	aBc aBc	aBc abc
Abc	Abc ABC	Abc AbC	Abc aBC	Abc abC	Abc ABc	Abc Abc	Abc aBc	Abc abc

On this supposition some of the whites must contain *m.* in a latent (or partly latent) condition—Abc; others contain *p.* completely latent—aBc;

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* 'Annals of the Royal Botanic Gardens, Peradeniya,' vol. 2, p. 333.

† Subsequent experiments showed that the non-appearance of the "ghost" maple did not necessarily imply the absence of A. The degree of development of this faint brown pigmentation on the testas of plants with white flowers seemed to depend to some extent on the manner of ripening, and to vary from complete invisibility up to a fair degree of brightness, and this sometimes in different seeds of the same plant.

others again contain both *m.* and *p.*—ABc. Finally, one white out of 16 contains neither—abc.

It seemed so certain that this was the true explanation that, in spite of the fact that no case of the kind had then been worked out, I ventured to predict, in the summer of 1904, that, when crossed with the simple grey type, some of these whites would yield plants bearing purple spotted seeds—*p.g.*; others seeds with the full brown marbling—*m.g.*; others again with both—*m.p.g.*; whilst some I expected to give only grey, like the grey parent used to render visible the hidden factor, if present.

The actual crosses were carried out in 1905 upon plants of the fourth generation (derived from heterozygotes in F_2) with the result set down in Table I.

One or more plants were raised successfully from each of 62 crosses, one of the parents having in each case exhibited grey testas and the other white (with or without the "ghost" maple). Each cross is entered twice, once according to the presence or absence of the purple character among the offspring, and once according to the presence or absence of the full maple character.

Considering first the purple character, nine of the white-flowered plants examined are found to have been heterozygotes in respect of the hidden factor, whilst 51 plants gave rise to offspring showing only the dominant or only the recessive character. The offspring were too few in number to allow of exactly determining how many of the parents were truly homozygous, but it is clear that a considerable majority must have been of this nature. The Mendelian expectation in the second generation of descendants from a heterozygote is a ratio of 3 homozygotes to 1 heterozygote.

In the case of the presence and absence of the maple character the result was closely similar. Thus the latency of *m.* and of *p.* in white flowered plants is demonstrated on a considerable scale, and the proportions in which they appear is not inconsistent with precise Mendelian segregation.

A similar result was obtained in the case of the offspring of a cross made by Mr. Bateson between Maple (*m.g.*) and Victoria Marrow (*w.*). On learning that I was engaged upon the experiments already described, Mr. Bateson very kindly handed over to me the crop obtained from this cross in F_2.

In F_1 the maple character was described as being simply dominant. In F_2 there appeared, *m.g.* 38, *g.* 12, *w.* 19, which is very close to the expected ratio of 9 : 3 : 4. Some of the seeds produced by two of the white flowered plants showed just the faintest possible trace of "ghost" maple.

In this particular instance the "ghost" maple made its appearance, even in later generations, in only a small number of those plants which could be shown to bear the latent factor for maple marking; *m.* is in fact almost, but not quite invariably, completely latent.

Offspring of all the 19 white plants were crossed with members of various pure strains of grey and purple seeded peas, in all of which maple marks were wanting. Table II shows that seven of the F_3 plants examined were heterozygous in respect of the hidden factor, whilst 22 yielded only dominant offspring, and seven only recessive.

In the light of these experiments it becomes extremely probable that the hidden factor—the cryptomere—comes always from the white flowered parent and not from the coloured, as Tschermak supposed* in the case of several pea crosses where reversion occurred in F_1, followed by the appearance of the ratio 9 : 3 : 4 in F_2.

To the behaviour of the maple character as thus described I know of no exception. The purple character, on the other hand, does not, as it would appear, invariably follow the simple rule obeyed by this character as exhibited by the coloured native pea of Ceylon. Hitherto among extracted whites derived from the cross between the Purple Sugar Pea and different white strains I have been able to find no example which does not yield exclusively purple offspring when crossed with grey.

Table I.

Note.—57. 1, 57. 2, etc., indicate different plants of F_4 derived from seeds of the same plant (No. 57) of F_3. It will be seen that some such families include all dominant members, others all recessive, and others again a mixture.

F_4. White parent number.		Coloured parent.	F_5.	
			p. present.	*p.* absent.
58. 6	×	*g.*	2	2
62. 2	×	*g.*	3	3
63. 1	×	*g.*	1	4
64. 10	×	*g.*	3	4
65. 2	×	*g.*	2	1
65. 6	×	*g.*	2	2
65. 8	×	*g.*	2	3
66. 4	×	*g.*	1	2
66. 8	×	*g.*	5	2
9			21	23

F_4. White parent number.		Coloured parent.	F_5.	
			m. present.	*m.* absent.
57. 1	×	*g.*	2	2
57. 1	×	*g.*	1	1
57. 2	×	*g.*	1	1
57. 3	×	*g.*	1	4
57. 7	×	*g.*	1	4
63. 1	×	*g.*	3	2
5			9	14

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* 'Archiv für Rassen- und Gesellschafts-Biologie,' vol. 2, p. 672, 1905.

F_4. White parent number.		Coloured parent.	F_5. *p.* present.	*p.* absent.
57.1	×	*g.*	2	—
57.1	×	*g.*	4	—
57.2	×	*g.*	2	—
57.3	×	*g.*	5	—
57.5	×	*g.*	3	—
57.7	×	*g.*	5	—
57.10	×	*g.*	2	—
58.2	×	*g.*	1	—
60.1	×	*g.*	2	—
60.2	×	*g.*	4	—
60.3	×	*g.*	4	—
61.3	×	*g.*	5	—
62.4	×	*g.*	5	—
62.4	×	*g.*	2	—
62.7	×	*g.*	6	—
62.8	×	*g.*	1	—
63.6	×	*g.*	2	—
63.9	×	*g.*	3	—
64.2	×	*g.*	3	—
64.3	×	*g.*	5	—
64.4	×	*g.*	3	—
64.5	×	*g.*	6	—
64.9	×	*g.*	6	—
66.5	×	*g.*	5	—
66.9	×	*g.*	2	—
23				
58.3	×	*g.*	—	2
58.4	×	*g.*	—	2
58.5	×	*g.*	—	6
59.1	×	*g.*	—	1
59.2	×	*g.*	—	4
59.3	×	*g.*	—	4
59.4	×	*g.*	—	5
59.5	×	*g.*	—	3
59.6	×	*g.*	—	2
59.7	×	*g.*	—	6
59.8	×	*g.*	—	2
59.9	×	*g.*	—	3
62.1	×	*g.*	—	3
62.3	×	*g.*	—	4
63.4	×	*g.*	—	2
64.7	×	*g.*	—	3
64.8	×	*g.*	—	3
65.1	×	*g.*	—	2
65.3	×	*g.*	—	3
65.5	×	*g.*	—	7
66.2	×	*g.*	—	3
66.3	×	*g.*	—	4
66.6	×	*g.*	—	3
66.7	×	*g.*	—	4
69.1	×	*g.*	—	2
69.5	×	*g.*	—	4
69.6	×	*g.*	—	4
69.9	×	*g.*	—	4
28				

F_4. White parent number.		Coloured parent.	F_5. *m.* present.	*m.* absent.
63.4	×	*g.*	2	—
63.9	×	*g.*	3	—
64.2	×	*g.*	3	—
64.3	×	*g.*	5	—
64.4	×	*g.*	3	—
64.5	×	*g.*	6	—
64.7	×	*g.*	3	—
64.8	×	*g.*	3	—
64.9	×	*g.*	6	—
64.10	×	*g.*	7	—
65.1	×	*g.*	2	—
65.2	×	*g.*	3	—
65.3	×	*g.*	3	—
65.5	×	*g.*	7	—
65.6	×	*g.*	4	—
65.8	×	*g.*	5	—
66.2	×	*g.*	3	—
66.3	×	*g.*	4	—
66.4	×	*g.*	3	—
66.5	×	*g.*	5	—
66.6	×	*g.*	3	—
66.7	×	*g.*	4	—
66.8	×	*g.*	7	—
66.9	×	*g.*	2	—
69.1	×	*g.*	2	—
69.5	×	*g.*	4	—
69.6	×	*g.*	4	—
69.9	×	*g.*	4	—
28				
57.5	×	*g.*	—	3
57.10	×	*g.*	—	2
58.2	×	*g.*	—	1
58.3	×	*g.*	—	2
58.4	×	*g.*	—	2
58.5	×	*g.*	—	6
58.6	×	*g.*	—	4
59.1	×	*g.*	—	1
59.2	×	*g.*	—	4
59.3	×	*g.*	—	4
59.4	×	*g.*	—	5
59.5	×	*g.*	—	3
59.6	×	*g.*	—	2
59.7	×	*g.*	—	6
59.8	×	*g.*	—	2
59.9	×	*g.*	—	3
60.1	×	*g.*	—	1
60.2	×	*g.*	—	4
60.3	×	*g.*	—	4
61.3	×	*g.*	—	5
62.1	×	*g.*	—	3
62.2	×	*g.*	—	6
62.3	×	*g.*	—	4
62.4	×	*g.*	—	5
62.4	×	*g.*	—	2
62.7	×	*g.*	—	6
62.8	×	*g.*	—	1
63.6	×	*g.*	—	2
27				

Table II.

F$_3$. White parent number.		Coloured parent.	F$_4$.	
			m. present.	*m.* absent.
1. 1	×	*p.*	2	3
5. 2	×	*p.*	2	1
5. 2	×	*g.*	—	1
5. 4	×	*g.*	2	2
12. 4	×	*p.*	1	4
12. 8	×	*g.*	2	2
12. 9	×	*p.*	1	2
13. 8	×	*p.*	6	1
7			17	15
1. 6	×	*g.*	1	—
5. 9	×	*g.*	3	—
8. 5	×	*p.*	1	—
8. 7	×	*p.*	3	—
9. 1	×	*p.*	5	—
9. 4	×	*g.*	2	—
9. 8	×	*g.*	3	—
10. 1	×	*p.*	7	—
10. 5	×	*g.*	3	—
10. 7	×	*p.*	5	—
10. 8	×	*p.*	2	—
11. 1	×	*p.*	1	—
11. 3	×	*g.*	1	—
11. 6	×	*g.*	6	—
12. 1	×	*g.*	5	—
12. 1	×	*p.*	3	—
12. 2	×	*g.*	6	—
12. 3	×	*p.*	6	—
14. 3	×	*p.*	3	—
14. 7	×	*g.*	5	—
14. 9	×	*g.*	4	—
14. 10	×	*g.*	2	—
21				
1. 3	×	*p.*	—	4
1. 9	×	*g.*	—	3
3. 4	×	*p.*	—	3
4. 1	×	*p.*	—	4
5. 1	×	*p.*	—	4
13. 5	×	*g.*	—	6
13. 7	×	*g.*	—	2
7				

On the Occurrence of Encystation in Trypanosoma grayi *Novy, with Remarks on the Method of Infection in Trypanosomes generally.*

By E. A. Minchin, M.A.

(Communicated by Professor E. Ray Lankester, F.R.S. Received November 3,—
Read November 8, 1906.)

In a tsetse-fly dissected and examined by me in the laboratory of the Sleeping Sickness Commission at Entebbe, Uganda, it was found that not only was the intestine swarming with *Trypanosoma grayi*, but the proctodæum also contained vast numbers of trypanosomes. Under moderate magnification they could be seen in dense clumps attached to the wall of the proctodæum, each clump having a superficial resemblance to a patch of mould, the whole mass, however, vibrating with the movements of the flagella. It was rather uncommon to find these parasites in the hind gut, and I at once made smears from different regions of the digestive tract, and carefully preserved and stained them. As it was but a short time before my departure from Entebbe, I was not able there to do more than glance at my preparations, but I noticed at once an important fact, which since my return to England I have been able to confirm and extend by careful study of my slides: namely, that in the hind gut the trypanosomes are in process of becoming encysted. Before proceeding to describe the encystment, I will say a few words about the conditions of the occurrence of these trypanosomes, and the manner in which they were preserved.

The tsetse-fly in question was one of a batch caught by our fly-boys at Entebbe on November 2, and fed the next day on a monkey infected with *Trypanosoma gambiense* from the cerebro-spinal fluid of a Sleeping Sickness patient. On every subsequent day these flies were fed on a healthy guinea-pig, and a certain number of flies were dissected daily and examined, until the batch was used up. Without going into details, it is sufficient to say that *T. gambiense* was found sparingly in the flies dissected up to 96 hours, that is to say, on November 4, 6, and 7. After this date *T. gambiense* disappeared completely and could not be found in any of the flies dissected. The fly in which the encystment was discovered was dissected on November 14. It must, of course, have been infected with *T. grayi* when caught, and, as I have stated, it had been kept 11 days in the laboratory and fed daily. At the autopsy it was found to be gorged with blood, and with all its organs perfectly normal and healthy in appearance. I examined the salivary glands and genital organs (testes and vesiculæ seminales) without finding anything resembling a trypanosome. Finally the digestive tract was examined, with the results stated. No trypanosomes were found in the proventriculus.

The films were made in the usual way by drying the smears, which were then fixed with methyl alcohol and stained with Giemsa's stain. A few were kept unfixed and have been fixed and stained for me recently by my assistant, Dr. J. D. Thomson, with excellent results. It is a matter of great regret to me now that none of my smears of this fly were fixed by the osmic-vapour method. The method of drying smears is a drastic one, which is likely to deform finer details, and in the present instance the cyst-wall is often injured, being evidently of a soft consistence.

The gut was divided into four regions and smears made from each. In the anterior region of the intestine the ingested blood is of red colour, very thick, and jelly-like and difficult to smear out nicely. Further back it gets more fluid and begins to turn black. In the hinder part of the intestine the blood is black and fluid. I refer to these three regions briefly as the red, red-black, and black blood respectively. The black blood stops sharply and suddenly at the point at which the Malpighian tubules enter the gut. The proctodæum contains no blood, but only a yellowish fluid containing innumerable coarse granules.

I proceed now to describe briefly the process of encystation observed by me in the fly. A glance at the preparation shows many different stages of the process side by side. In the first place we find individuals in which encystment has not begun (figs. 1, 2). These are forms for the most part very slender and smaller than any of the forms of *T. grayi* found in the intestine of this or other tsetse-flies; but their most striking feature is the absence of any distinct undulating membrane, so that they bear a great resemblance to the genera *Herpetomonas* and *Crithidia*, especially the latter. The flagellum is long and appears to run down the side of the body. The blepharoplast has the large size and rod-like form characteristic of *T. grayi*. The nucleus is either compact or broken up into granules of chromatin. Division stages have been found, but are very rare.

In the first stages of encystment the flagellum becomes shortened and stains more deeply, suggesting that as it diminishes in length it becomes thickened. At the same time the cyst begins to appear as a layer of substance which stains reddish with Giemsa, forming a cap at the hinder pole (figs. 3, 4). These two processes continue until on the one hand the flagellum is completely retracted and on the other hand the body is enveloped in a pear-shaped cyst, at first incomplete towards the pointed end (figs. 5, 6). The flagellum appears to become retracted into a pink-staining vacuole (fig. 5, *fl. v.*), which reminds one of the flagellar vacuole described by Leishman during the formation of a flagellum in the Leishman-Donovan bodies in cultures, but here the sequence of events is inverted, as it were. Finally the pink vacuole disappears, but a streak, which gradually fades away, can be seen for a time in front of the blepharoplast (fig. 6). The cyst closes up round the pointed end of the body, and then changes in form, becoming first oval (fig. 7) and then irregularly circular in outline (fig. 8). In the circular cysts, which are the last stage, it is difficult to make out much detail, but in favourable examples they can be seen to contain hyaline protoplasm, staining faintly bluish, with the dark purple-stained blepharoplast and the nucleus generally represented by irregularly scattered granules, staining a purplish-red tint. The cyst-wall also stains strongly and appears slightly redder than the nucleus.

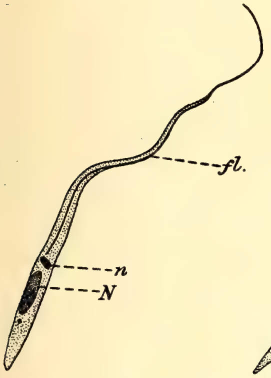

Fig. 1.

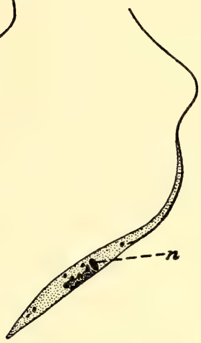

Fig. 2.

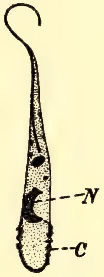

Fig. 3.

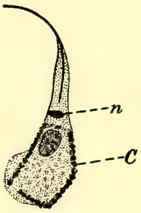

Fig. 4.

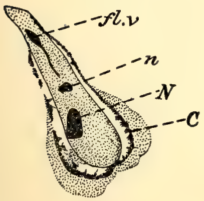

Fig. 5.

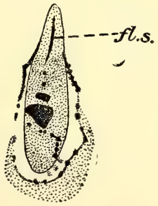

Fig. 6.

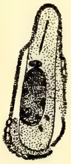

Fig. 7.

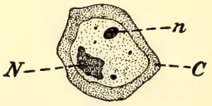

Fig. 8.

I will now state briefly the conditions observed in other parts of the gut of the fly in which the cysts were found. The red blood was found teeming with trypanosomes, nearly all large forms of indifferent type. Very few young forms were found, and male forms were very scarce. No pronounced female types were found in this region, though many of the large indifferent forms approach the female type in the shortness of their free flagellum. In the red-black blood the conditions were similar, but young forms are becoming commoner. In the black blood young forms predominate, and large individuals are comparatively infrequent. Thus in the gut of this fly as a whole we find great rarity of differentiated sexual forms, but a swarm of indifferent forms which in the hinder part of the intestine give rise to very numerous young forms, and these in their turn would appear to pass into the small, *Herpetomonas*-like forms found in the proctodæum, starved-looking creatures which, in a medium where there is probably no nutriment, go through the process of encystment already described.

Having so far confined myself strictly to matters of fact, I will now offer a few suggestions and speculations as to the probable significance of the encystation in the economy of the life-cycle of the parasite. In the first place, the resemblance of these cysts, and especially the pear-shaped forms, to the "Schleim-Cysten" described by Prowazek* in *Herpetomonas muscæ-domesticæ,* Burnett, is very marked, and has struck everyone to whom I have shown both my preparations and Prowazek's figure. I think there can be no doubt that they are similar bodies, and have a similar function, that is to say, that they are destined to pass out of the gut of the fly with its dejecta. Very numerous analogies in support of this inference could be cited from other parasitic Protozoa. The question which interests us most is: what becomes of them after being cast out from the fly? In the absence of any observations or experiments upon this point, one can only draw conclusions from the analogy of what is known in other cases. In the case of *H. muscæ-domesticæ* the cysts are scattered about everywhere, and, as house-flies are far from being particular in their feeding or sanitary in their habits, a fly runs every chance of infecting itself with the *Herpetomonas* by swallowing with its food cysts dropped by another fly. There can be no doubt whatever, it seems to me, that this is the manner in which the house-fly acquires the infection of the *Herpetomonas.* The case is, however, very different with the tsetse-fly, which does not haunt the enclosed spaces and insanitary surroundings of the house-fly, but lives a free, open-air life by lake and forest. Nor is the tsetse-fly a foul feeder like the house-fly. My assistant, Mr. Degen, and myself made many attempts to feed tsetse-flies on all kinds of food, but always without the least success. On the other hand, the tsetse-fly is a greedy blood-sucker, and will attack anything from a frog, lizard, or bird to a hippopotamus, but, in my opinion, it does not feed in any other way. Further, the tsetse-fly is not like the *Stomoxys,* which lays its eggs in dung; it is viviparous, as is well known, and nourishes its larva in the uterus until full-grown.

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* 'Arbeiten a. d. k. Gesundheitsamte,' Berlin, vol. 20 (1904), p. 446.

For all these reasons it seems to me in the highest degree improbable, indeed I may say impossible, that a tsetse-fly would ever infect itself by sucking up cysts dropped by another fly, or that a parasite which had to depend on this method of dissemination could maintain its existence in the tsetse-fly. The only possible destiny I can imagine for these cysts is to be swallowed accidentally by some vertebrate, the (as yet unknown) host of *Trypanosoma grayi*, in order to germinate in its digestive tract, to pass thence into the blood, and to be taken up again with the blood by the tsetse-fly. A cycle of this type is as yet unknown, but there are abundant analogies for all parts of it. In the first place, it is a common thing for animals to have protozoan parasites in the gut, which they take up in the encysted condition after they have been dropped by another individual. Without multiplying instances unnecessarily, I may point out that Schaudinn proved the infection of *Amœba coli* to originate in this way, and that it is a common human parasite in regions where sanitation has not advanced beyond the primitive condition of *épandage par terre.* In the second place, there are many instances among Sporozoa of cysts germinating in the intestine and liberating motile forms which then pass through the wall of the gut into other organs of the body.

In a former communication by my colleagues,* Lieutenants Gray and Tulloch, and myself, we were able to confirm Bruce's results as to the existence of direct mechanical infection by means of the tsetse-fly, which, if it stabs its proboscis first into an infected animal and then soon after into a healthy one, can infect the latter. We were not able to demonstrate, however, what I may term cyclical infection, which at present has not been shown to exist. I suggest that there are two possible modes of cyclical infection, in the dissemination of protozoan blood-parasites by biting insects generally. In one method, which I may term *inoculative*, the parasite, after going through developmental changes in the insect, passes back again into a second vertebrate host through the proboscis, as in the case of malaria transmitted by a mosquito. In the other method, which I propose to term *contaminative*, the parasite taken up by the biting insect, after going through developmental changes within its gut, would pass out of it through the anus, and infect the vertebrate host by contaminating its food or drink. We have all of us (I speak for myself) been imbued hitherto with the idea that the cycle of the trypanosome in the tsetse-fly must be of the inoculative type, and have failed to find it. I wish to suggest strongly to those working on the subject of trypanosome-infection the desirability of making experiments and observations to prove or disprove the existence, in the insect which disseminates the parasite, of a life-cycle which results in a contaminative infection of the vertebrate host.

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* 'Roy. Soc. Proc.,' B, vol. 78 (1906), p. 242.

A full account of the trypanosome-cysts described in this note, and of other points relating to *Trypanosoma grayi*, will be published, with illustrations, in the 'Quarterly Journal of Microscopical Science.'

DESCRIPTION OF THE FIGURES.

Figs. 1 and 2.—Free-swimming *Herpetomonas*-like forms of *Trypanosoma grayi* from the proctodæum of the tsetse-fly, before encystation has commenced; *N.*, principal nucleus; *n.*, blepharoplast; *fl.*, flagellum. × 2000.

Fig. 3.—Commencement of encystation. The flagellum is becoming retracted, and the first appearance of the cyst-secretion is seen at *C.* × 2000.

Fig. 4.—Similar stage, slightly more advanced. The cyst-wall (*C*) is damaged at one point. × 2000.

Fig. 5.—Flagellum completely retracted, represented by a vacuole (*fl. v.*). × 2000.

Fig. 6.—The flagellar vacuole is absorbed and the flagellum is represented only by a delicate streak (*fl. s.*). Cyst much damaged. × 2000.

Fig. 7.—The cyst-secretion now extends all round the body. × 2000.

Fig. 8.—Rounded-off cyst, the final stage. Letters as before. × 2000.

On the Transpiration Current in Plants.

By Henry H. Dixon, Sc.D., Professor of Botany in Dublin University, and Director of Trinity College Botanic Gardens.

(Communicated by Professor J. Joly, F.R.S. Received November 7,—Read December 6, 1906.)

In a paper* published in 1905, Professor Ewart investigates the question as to what force is required to move water through the waterways of plants at the same velocity as the transpiration current. His general conclusion is that the resistance is so great that neither are sufficient forces generated in the leaves to raise the water at the required velocity, nor is the tensile strength of water adequate to transmit these forces downwards, if such existed. In a subsequent paper he seems to have modified this latter view and quotes Berthelot as stating that air-free water may support a tension of over 200 atmospheres.†

It is needless to criticise Ewart's calculations of the resistance based on Poiseuille's formula, which he himself admits is quite inapplicable to the case, owing to the presence of cross-partitions and irregularities in the cross-sections of the tracheal tubes. It may be noticed, however, that when care was taken that discontinuities were not present in the water columns of the wood experimented upon, the flow observed approximated to the flow calculated by the formula. In an experiment on a piece of yew wood the approximation was very remarkable. The actual amount transmitted‡ through a length of 15 cm. was 4·2 c.c. per hour, while the calculated amount was 9·8 c.c. The average distance from one another of the cross-partitions in the fine tubes composing the wood is 0·25 cm. Therefore about 60 partitions must be traversed in passing through a length of 15 cm. This indicates that the resistance offered by the walls, or rather by the pits in the walls, to the passage of water is very slight.

Ewart also endeavours to find experimentally the resistance offered to the transpiration current. His first method, that of Janse and Strasburger,§ was as follows: "Leafy branches 4 to 8 feet in length were cut under water and kept in darkness for half an hour. Clean ends were then cut under water, placed in freshly filtered eosin solution, and at once exposed in the open on bright cloudless breezy June days between 10 a.m. and 1 p.m., with a shade temperature averaging 18° to 22° C. The conditions for transpiration were, therefore, optimal.

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* "Ascent of Water in Trees," 'Phil. Trans.,' 1905.

† "The Resistance to Flow in Wood-vessels," 'Ann. of Botany,' vol. 19, No. 75, July, 1905.

‡ "Ascent of Water in Trees," 'Phil. Trans.,' 1905, p. 50.

§ Strasburger, 'Histologische Beiträge, 1901,' Part III, p. 778.

"After a timed period the stem was removed and rapidly sectionised from apex downwards until the eosin solution was visible in the wood, the length of the remaining portion of the stem giving the rate of flow during the period of observation. Portions of the same stems and also similar ones from the same plants were then subjected to varying water pressures until closely corresponding rates of flow were reached."*

By this method, and by assuming that the velocities shown in these branches are maintained throughout the stem, Ewart obtains results which indicate that, in order to move water in the stems of plants at the velocity of the transpiration current, pressures equivalent to a head of from 6 to 33 times the height of the plant are required.

There are several reasons why this unexpected result of Ewart's must be regarded as incorrect. (1) The velocity of flow given in Ewart's experiment is probably far in excess of even the maximum velocity of the transpiration current in the intact plant. (2) The velocity cannot be assumed to be uniform throughout high trees; but may fall off from below upwards. (3) Ewart's results for the resistance to flow in stems are not in agreement with a large body of experiment to be quoted presently, but appear to be excessive.

First, with regard to the velocities in Ewart's experiment and in intact trees. In intact trees the lifting forces generated in the leaves must do work against the resistance to flow all along the path of the current, and, if the supply is inadequate from the roots, against other opposing forces in addition, and against the whole hydrostatic head. In Ewart's experiment not only are the resistance of the lower part of the conducting system, the other opposing forces and the hydrostatic head removed, but they are replaced by the atmospheric pressure acting as a *vis a tergo*, urging the water upwards. Naturally, then, a much greater velocity is attained in the latter case than when the branch is still attached to the tree.†

The subsequent wilting of the leaves of cut branches shows that clogging afterwards greatly reduces the flow; but, of course, in the experiment quoted it is only the initial stage which is recorded.

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* Ewart, *loc. cit.*, p. 24.

† It may be mentioned that Janse's method of observing the amount of transpiration by successive weighings of a severed branch is not falsified by this error, but probably gives too small an amount after transpiration has proceeded for some time. Strasburger considers that this is corrected more or less accurately by his over-estimate of the resistance (Strasburger, *loc. cit.*, p. 779).

The assumption that the velocity in the terminal branches is as great or greater than in the trunk is also not justified. The lower leafy branches have to do work against a smaller hydrostatic head, and against a smaller resistance than the outer and upper branches, and consequently the flow will be faster through the lower parts than above. Furthermore, in many cases the effective cross-section supplying unit transpiring area is greater above than below. A good instance of this was brought under my notice by Dr. J. Joly last summer, in a young specimen of *Abies excelsa,* which had just been felled. The tree was 550 cm. high. The section at its base showed 17 year rings. The lateral branches were almost uniformly clothed with leaves, so that the length of the branches was approximately proportional to the leaf-area it supported. At 52 cm. from the apex the area of the cross-section of the wood was 0·63 sq. cm. and the sum of the length of the leafy branches above this was 170 cm. At 134 cm. from the apex the cross-section was 4·29 sq. cm. and the sum of the lengths of the leaf-bearing branches above was 1500 cm. At the higher level the cross-section of the supply conduits was 0·37 sq. cm. per 100 cm. of leafy branch, at the lower point the cross-section of the supply was 0·286 sq. cm. per 100 cm. of branch. Therefore, in the case of this tree, if all the leaves were transpiring uniformly the velocity at the lower level must be greater than above. Hence in Ewart's experiments it is quite possible that the velocity below was considerably in excess of that in the upper parts of his severed branch, and certainly without special measurements it is not legitimate to assume that the velocity throughout the length of the branch was uniform. This is equally true with regard to intact large trees.

With regard to the resistance it will be seen later on that Ewart's results are considerably too high.

The second method of determining the maximal velocity of the transpiration current employed by Ewart* is also open to objection. It is described by him as follows: A small branch bearing a small number of leaves, while still attached to the tree, is led through a split rubber cork into an air-tight glass chamber containing a weighed quantity of calcium chloride. The gain in weight of the latter gives the amount of water transpired by the leaves in a given time. Assuming, then, that all the leaves of the tree may act like those in the closed glass chamber, the number of leaves on the whole tree will give the weight of water transpired by the tree and consequently the amount of water which passes up the tree in a given time. Then, by measuring the effective cross-section of the trunk, the velocity of the transpiration current may be estimated.

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* *Loc. cit.*, p. 56.

The objections to this method are obvious.

1. When the branch is first introduced into the desiccated chamber it will lose water more rapidly than when it was transpiring into the less dry external air. It will continue this abnormally rapid rate of transpiration until the concentration of the vacuoles of the evaporating cells reaches a steady state, depending on the freedom of supply from the water conduits and the vapour pressure in the chamber, but, until this steady state is attained the amount of water entering the calcium chloride may be largely in excess of that passing up the stem.

2. But a more serious source of error is the assumption that all the leaves of the tree can transpire at the same rate as those on the single desiccated branch, whereas the supply, which under the conditions of the experiment is available for the very actively transpiring branch, would be largely encroached upon if all the branches were under equally favourable conditions for transpiration. In fact, the single branch in the desiccated chamber and still attached to the rest of the tree, which is under normal conditions of moisture, is under conditions of supply approximating to those of a cut branch set in water and, for the same reasons, cannot be assumed to give a correct estimate of the velocity of the transpiration current throughout the whole tree.

The validity of this objection may be demonstrated experimentally by weighing the amount of water given off by a given number of leaves in a desiccated chamber, and comparing this amount with the quantity of water transpired by the same number of leaves on the same tree exposed to normal conditions of maximal transpiration.

I have made several of these experiments: A small yew-tree was removed from the flower-pot in which it had been grown, and the roots, and their surrounding soil, enclosed in a rubber bag; to prevent loss of water, except from the leaves, the opening of the bag was tied tightly round the stem. Periodic weighings gave the amount of water transpired. At the same time a branch still attached to the tree was introduced into a hermetically closed flask containing calcium chloride. The flask could be removed and weighed periodically. A rubber bag, similar to that enclosing the roots, filled with moist earth and closed, was exposed to the same conditions and weighed simultaneously, thus giving a small correction for loss through the bag. In one of these experiments the unenclosed branches supported approximately 9500 leaves, the enclosed branch 520, *i.e.*, the proportion of leaves on the single branch to those on the whole tree was 1:18. When the tree was exposed in a hot sun and brisk breeze, air temperature varying between 31° and 24° C. in the month of July, the amount transpired was 5·171 grammes per hour, or 0·544 gramme per 1000 leaves. The amount transpired by the desiccated branch, on the other hand, was 0·781 gramme per hour, or 1·502 grammes per 1000 leaves per hour. Hence the leaves in the desiccated chamber transpired nearly three times as much as the others under normal conditions of maximal transpiration. In this experiment the loss of water from the enclosed branch does not represent any temporary desiccation of the surface tissues, for before the weighings were made the branch had been enclosed in the flask for a day. If this initial desiccation had been included, as it was in Ewart's experiments, the difference between the amounts given off by the enclosed and unenclosed leaves would have appeared greater.

In diffuse light the difference is not so marked. The unenclosed leaves of the tree used in the last experiment in diffuse light with a temperature of 21° C. transpired 1·250 grammes per hour, or 0·130 gramme per 1000 leaves; the enclosed leaves simultaneously transpired 0·099 gramme, or 0·192 per 1000 leaves per hour. The desiccated leaves are nearly $1\frac{1}{2}$ times as active in transpiration as those under the normal conditions.

These experiments show that it is not justifiable to assume that the rate at which water is given off by an isolated branch under conditions of abnormal desiccation is attained by all the branches when all alike are exposed to conditions most favourable to transpiration. The excess evaporation from the desiccated leaves will be greater when the bulk of the isolated branch is but a small fraction of the bulk of the whole tree; for the greater the preponderance of the latter the larger will be the supply available for the branch and consequently the less the resistance to transpiration. In Ewart's experiment, then, where the branch had only 500 leaves, while the whole tree had 9,000,000 (1:18,000), it is probable that the effectiveness of the former was much greater than that of the remaining leaves of the tree. Such an over-estimate in the amount transpired involves, according to the method, an exaggeration in the velocity of the current in the trunk.

The control of transpiration exercised by the freedom of supply may be easily observed by means of the weighing method. The amount transpired will be found to fall off as the plant exhausts the water in the soil round its roots, and to rise when the soil is again rendered moist. The following figures illustrate this fact in the case of a small yew-tree which was exposed to conditions favourable to transpiration on seven successive days. The conditions were fairly uniform, as throughout the experiment the sky was lightly overcast and a light east wind blew.

Table I.

Transpiration per hour.	Date.	Temperature.
grammes.		° C.
2 ·65	July 24	18 ·5
2 ·14	„ 25	20 ·5
3 ·25	„ 26	22 ·0
0 ·87	„ 27	19 ·5
0 ·97	„ 28	21 ·5
Watered—		
2 ·23	„ 28	24 ·0
Watered—		
1 ·59	„ 30	19 ·0
3 ·05	„ 30	22 ·5

The dependence of transpiration on the supply is prettily illustrated by Darwin's experiment with the horn hygroscope.* Darwin records that when the hygroscope is applied to the leaves of a branch severed from a plant, it indicates a gradually diminishing rate of transpiration as the store of water in the branch is gradually exhausted. A diminished rate of transpiration is also indicated by the hygroscope when the supply to a branch is reduced by the application of a clamp constricting the water conduits leading to its leaves.

The diminution in the rate of transpiration in these experiments is no doubt due to a rise in the tension of the water columns supplying the evaporating cells. The tension rises in the first case as the limited water store is drawn upon, and the water surface is dragged into the cut surface of the branch. The surface-tension forces developed there then oppose the transpiration current. In the second experiment the tension is increased by the rise of the resistance brought about by the reduction of the calibre of the conduits. Further reduction of transpiration is occasioned by the closing of the stomata and by other phenomena, which are themselves direct or indirect consequences of the rise of tension in the water.

According to this view an interesting observation of Darwin's† receives a ready explanation. There is a momentary increase of transpiration in these experiments immediately after the separation of the branch and immediately after the application of the clamp. In the first case we may assume that the tension in the water supply is reduced by fracture, in the second case by the compression applied when screwing up the clamp. Transpiration obviously soon re-establishes tension, which becomes greater in both cases than it was originally.

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* Darwin, "Observations on Stomata," 'Phil. Trans.,' B, 1898, pp. 539 ff.

† Darwin, *loc. cit.*, pp. 548 ff.

In many ways, then, we see it is established that supply largely controls transpiration, and in neglecting this factor Ewart considerably exaggerated the maximal velocities of the transpiration through the stems of trees.

We come now to consider the amount of resistance experienced by the transpiration current in its passage through the water conduits.

To obtain the resistance, Ewart forced water through lengths of branches under various pressures. The pressure which gave the same velocity in the branch as that estimated by his methods for the transpiration current he took to be equivalent to the resistance experienced by the transpiration current. Ewart does not mention how he prepared the pieces, or how he cleansed the water for the experiment. The introduction of air-bubbles or of any clogging substance at the cut surface would materially exaggerate the pressure needed to obtain the observed velocity. Indeed, if the branch were transpiring actively when the experimental pieces were removed, it would not be sufficient to cut it under water; for the liquid in the branch being in tension, bubbles would be formed at the surface of the cutting knife. These bubbles would require some time to dissolve and disappear. The slimy materials exuding from the injured cells also clog the branch and raise the apparent resistance of its conduits. The comparatively high pressures with which Ewart worked would render the clogging from this source and from any impurity in the water more marked. Fig. 1 illustrates this point. The

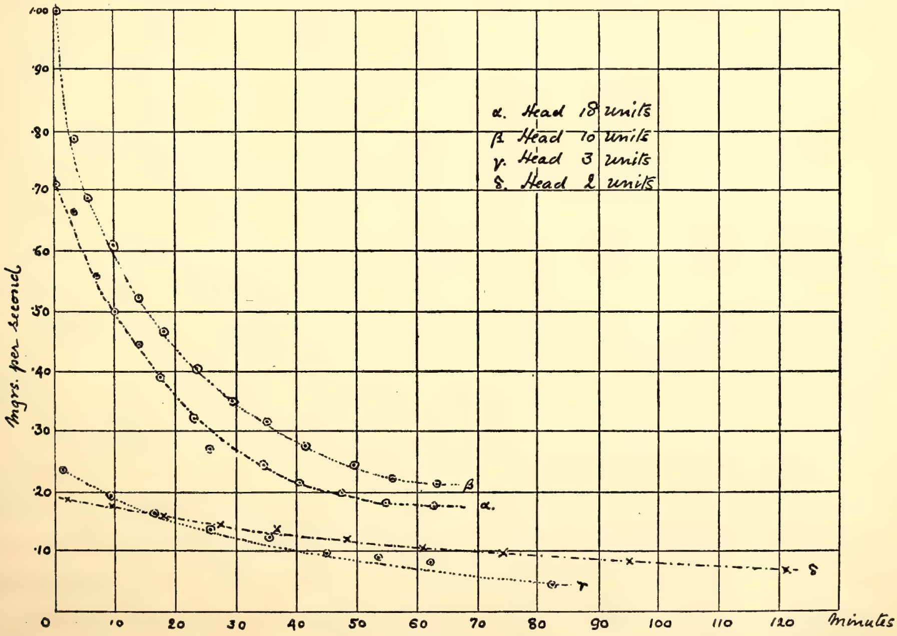

Fig. 1.

ordinates of the curves there shown indicate weights of filtered tap-water transmitted through 3 cm. of wood of *Abies pectinata* per second. The abscissæ indicate time in minutes. The curves show the diminution in the rate of transmission for each pressure, the fall-off in the amount being much more rapid for the higher pressures. From these it is seen that it is desirable (especially when using colour solutions, which from the nature of the case cannot be distilled) to employ low pressures in order to determine the resistance of the conduits apart from the surface resistance. For this reason, then, when I began to determine the resistance of the tracheidal tubes I preferred to experiment with very low pressures, assuming that the flow for higher pressures would be proportional to the head. This point was examined later.

The wood used in these experiments was that of *Taxus baccata.* The uniformity and comparative homogeneity of structure (resulting from the fact that it is composed solely of tracheids and is not penetrated by resin ducts) recommend it as far the most suitable wood for experiment, when the qualities of the water-conducting tissues are being examined. The tracheids composing the wood of *Taxus baccata* are elongate spindle-shaped chambers 1 to 5 mm. long and approximately square in cross-section. The cavity of these chambers is comparatively small. In cross-section the lumen forms about a quarter the entire area of the tracheid. The only other constituents of the wood are the cells of the medullary rays, which are radial tiers of cells 0·4 to 0·02 mm. in height. They do not materially differ in percentage from one level in the stem to another, and consequently do not introduce differences of serious magnitude. The fact, however, that they exude small amounts of slimy materials sometimes introduces difficulties. But inasmuch as there are approximately the same amount of medullary ray-cells per unit area in every cross-section, the clogging introduced in this manner is uniform and, it is to be noticed, tends to increase the apparent resistance. The presence in other woods of large medullary rays, of wood-parenchyma, of vessels of variable size, or even of resin ducts, renders them unsuitable for experiment.

Certain precautions are necessary in preparing the wood for these transmission experiments. It is best to cut away the transpiring leaves from the selected branch, and then to cut off a short length of it (say 15 to 25 cm.) under water. Five to ten centimetres are now cut from each end, and the remainder, after lying in water for at least 30 minutes, may be used directly or reduced to smaller lengths for experiment. These precautions are necessary, so that bubbles shall not be generated in the conduits. It seems possible that Ewart's high estimate of the resistance was, in part, due to bubbles being generated in the conduits while preparing the wood for his resistance experiments. He certainly does not mention having taken these or similar precautions in this connection, and his results are three to four times as high as those given when these precautions are taken.

When determining the velocity of flow with colour solutions, I usually worked with a head of water equal to, or less than, the length of the piece of wood experimented with. When using unit head, a fresh surface was cut with a razor on one end of a piece of a branch prepared as just described. The piece was supported vertically with the fresh surface uppermost, and a little vaseline smeared round the bark of that end. A drop of filtered concentrated eosin solution was placed on the upper surface, and as the drops formed at the lower end they were drawn away by lighly touching them with bibulous paper. As the eosin sank through the wood it was kept constantly replenished drop by drop above. After a definite time the experiment was stopped, and longitudinal cleavage of the piece of wood showed the distance travelled by the eosin solution during the time of observation at unit head. The following are the results of some of these observations:—

Table II.

Length of piece.	Time of observation.	Distance travelled.	Velocity per hour.
centimetres.	minutes.	centimetres.	centimetres.
7 ·2	15 ·0	1 ·7	6 ·8
6 ·0	10 ·0	1 ·2	7 ·2
4 ·0	15 ·0	1 ·7	6 ·8
3 ·5	15 ·0	1 ·8	7 ·2
10 ·0	20 ·0	2 ·2	6 ·6
3 ·0	25 ·5	3 ·0	6 ·9

In the last experiment the fluid transmitted was a solution of ferrocyanide of potassium. Its presence below was detected by ferric chloride. The mean of these experiments gives a velocity of 6·9 cm. per hour under a pressure equivalent to a head of water equal in length to the experimental piece of wood.

Experiments were also made at lower pressures. For these the water-pressure was applied at the lower end of a vertical piece of wood, through a rubber tube bent into a U-shape. This tube was filled with eosin solution and the surface of the solution was raised to the desired height above the upper surface of the wood.

The mean of these observations is 8·5 cm. per hour, calculated to unit head. If we exclude the two extreme observations we get 7·6 cm. per hour

Table III.

Length of piece.	Head.	Distance traversed.	Time.	Velocity per hour per unit head.
centimetres.	centimetres.	centimetres.	minutes.	centimetres.
8	2	0·7	20	8·4
6	2	0·8	20	7·2
6	3	1·8	15	14·4
6	3	0·9	15	7·2
6	3	0·7	15	5·6

as the velocity under a head equal in length to the stem. The last observation of the series was made on a piece of a narrow branch about 0·5 cm. in diameter, the others on pieces about 1 cm. in diameter. This difference appeared almost constantly in my experiments. The thin distal portions of the wood in almost every case offer a greater resistance to flow than the thicker parts. The high estimates of resistance are almost always obtained with the former. This fact is probably of importance in determining the total resistance in the intact plant. I have included the third observation in the table, although it diverges so markedly from the mean, because I could see no error in the experiment, and it is quite possible that a maximal result like this is the nearest to the actual flow in the uninjured tree.

The higher mean in the second series of experiments for the velocity of transmission is probably due to the fact that clogging substances are less likely to accumulate owing to the actually slower flow and to the position of the surface of application.

With care, good results may be obtained with higher pressures, if the supply is from below. In the following experiments (Table IV) the cylinder of wood was fixed in the short arm of a vertical J-tube filled with a repeatedly filtered solution of ferrocyanide of potassium. The moment of penetration through the wood, which was 3 cm. long in each case, was determined by its reaction with ferric chloride applied in a piece of bibulous paper to the upper surface of the cylinder.

The mean of the entire series gives 6·9 cm. per hour as the velocity at unit head. As all the known errors, such as the introduction of bubbles, clogging and injury of the tracheidal tubes, tend to reduce the result, it is probable that the velocity in the intact tree would be at least 7 to 8 cm. per hour under the same pressure. The occasional high results obtained indicate a still higher figure as the probable velocity.

In fig. 2 I have plotted these results. The ordinates represent the length traversed in one hour, while the abscissæ indicate the pressures, considering a head equal to the length of the branch as unity.

Table IV.

Head.	Time traversing 3 cm.	Velocity per hour at unit head.
centimetres.	minutes.	centimetres.
30	2·0	9·0
30	3·0	6·0
27	2·0	10·0
27	2·5	8·0
24	3·75	6·0
24	3·5	6·4
24	3·5	6·4
21	4·5	5·7
18	5·25	5·7
18	4·5	6·6
18	4·75	6·3
15	4·5	8·0
15	4·5	8·0
12	5·5	8·2
12	7·0	6·3
12	5·5	8·2
9	10·25	5·8
9	7·75	7·7
9	9·5	6·3
6	12·75	7·0
6	13·0	6·9
3	25·5	7·0

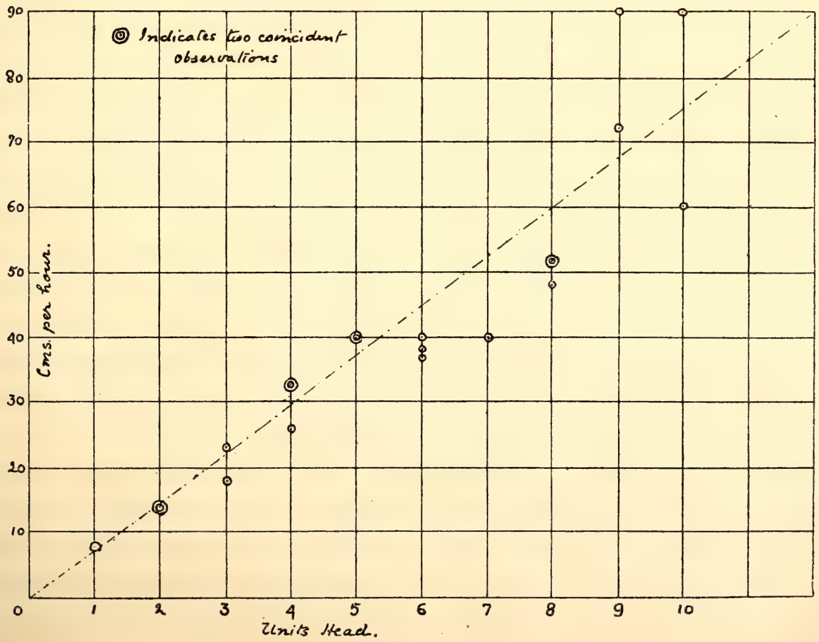

FIG. 2.

It may be noticed that the most divergent observations are those made at the higher pressures.

When we compare these results with Ewart's* a very wide discrepancy is apparent. The results of three of his experiments allow themselves to be readily compared with my figures.

(1) He found that water travelled in a piece of yew stem, 35 cm. long, at the rate of 11·7 cm. per hour under a head of 3 metres. The head here is nearly 8·6 times the length of the transmitting wood. Assuming the velocity proportional to the pressure, at unit head the water would travel at 1·36 cm. per hour. This rate is stated to be maximal.

(2) On pp. 51 and 52 it is stated that the rate of flow in a piece of yew wood, 25 cm. long, under a head of 3 metres is 26 cm. per hour. In this experiment the head is equal to a column 12 times the length of the transmitting branch; when reduced to velocity under unit head the result is 2·17 cm. per hour.

(3) Again, on p. 55 an experiment is recorded which is suitable for comparison. A velocity of 19 cm. per hour was observed in a branch 25 cm. long under a head of 4 metres. This becomes 1·19 cm. per hour under unit head.

The mean of these three observations gives 1·57 cm. per hour as the maximal velocity of flow in the yew wood under unit head.

The results of my own numerous observations, on the other hand, made under very various conditions of pressure and by different methods, point to a velocity exceeding 7 cm. per hour with the same head.

The only explanation of this discrepancy which appears possible is that in Ewart's experiments sufficient care was not taken to prevent bubbles forming in the opened conduits and to obviate clogging at the surface. This last effect would be exaggerated in his experiments, as he worked apparently in every case at such high pressures. Reference to fig. 2 illustrates this point. There it appears that the erratic observations are those made at high pressures, although at both high and low pressures similar precautions were taken. It is in only the first of Ewart's experiments quoted above that it is mentioned that the experimental branch was cut under water. Other precautions are not mentioned.

Whatever is the cause of the discrepancy, it is certain that if Ewart had obtained my results, the difficulty of resistance, which he finds to be fatal to the cohesion-theory of the ascent of sap, would not have presented itself to him, for the velocity of 7 cm. per hour, which he demands† in the stem of the yew, would not require a pressure equivalent to a head of 65 metres of water, as he supposes, but only to 11 metres. The recognition of the fact that unit head produces a velocity of about 7 cm. per hour invalidates his whole calculation, intended to show that to raise the sap in trees 150 metres high would require a pressure approaching 100 atmospheres. The facts of the case would be more correctly stated by saying that if the amounts transpired by isolated branches, under exceptionally favourable conditions for transpiration, were transpired by the remaining branches of the yew, and if we further assume that the velocity in the trunk is maintained out into the finest branches of high trees, then the resistance to flow in the conducting tracts would be about equal to a head of water the same height as the tree.

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* *Loc. cit.*, p. 49.

† *Loc. cit.*, pp. 56 and 57.

My results for the resistance, which were obtained as described above by directly measuring the velocity of flow under a given head, were fully confirmed by other experiments in which the amount transmitted under a given head was observed. An estimate of the cross-section effective in transmitting the current then gives the velocity.

A piece of a branch of *Taxus baccata*, 4 cm. long and having a woody cylinder 1·35 cm. in diameter, was placed with its long axis vertical. Water was supplied at its upper surface just as quickly as it percolated through the wood, so that the upper surface of the wood was continually wet, but the water was never appreciably piled upon it. The amount of water transmitted in this manner under unit head was 1·356 gramme per hour. In order to find the effective cross-section, after this observation was made, a solution of eosin was supplied under similar conditions. By this means the transmitting portions were coloured, and the area of their cross-section easily estimated. A mean of three such estimations gave the effective cross-section as 0·7 sq. cm. From this it appears that under unit head 1·93 grammes is transmitted per square centimetre per hour. Assuming with Ewart that the lumina occupy about 0·25 of the cross-section, the velocity to secure this rate must have been 7·5 cm. per hour. This agrees well with the results obtained with the other method.

In order to determine the amount of flow without danger from the error of clogging at the surface of supply, the flow was measured in a lateral branch springing from a stout stem. Water under pressure was supplied at the two cut ends of the stem. With this arrangement, owing to the relatively large surfaces of supply, the clogging taking place at these does not encroach upon the amount necessary for the supply of the small lateral branch for several hours, and consequently the rate of transmission in the lateral branch remains constant for several hours. To quote one of these experiments: A piece of yew stem 7·3 cm. long, having a straight lateral branch at its middle length, was selected. The diameter of the stem was 0·85 cm. and 1·05 cm. at each end respectively. Its wood was also laid bare by an oval scar where another lateral branch was removed. This scar was 1·3 cm. by 1·1 cm. The small lateral branch was cut to a length of 10·5 cm., and its wood had a diameter of 0·47 cm. Not more than a quarter of its cross-section was in a state suitable for transmitting water—the greater part being occupied by blackened duramen. This lateral branch was fixed water-tight in a rubber bung in an orifice in the bottom of a tank in such a manner that the cut end of the lateral branch projected from the tank, while the supporting stem was immersed in the water in the tank. With the head of 30 cm. 0·300 gramme were transmitted per hour. The transmitting cross-section was about 0·043 sq. cm. One-quarter of this area would be lumen: therefore the velocity of flow must have been 27·2 cm. per hour. If we reduce this to unit head the velocity becomes about equal to 9·4 cm. per hour, which again falls within the limits of the results obtained by the previous method.

Throughout this discussion it has been assumed that the velocity is proportional to the pressure. This is usually done. Fig. 3 shows that this is certainly approximately true for pressures up to 16 units head. On fig. 3 are plotted the results of many experiments, the object of which was to determine if the amount transmitted in a given time is proportional to the pressure, as the former assumption would involve. The utmost care was taken to obtain clean water to supply to the wood and to free the latter from clogging material as much as possible. The water used was carefully distilled, the vessels and tubes of supply were repeatedly washed with distilled water before using, and the surface of the wood freshly cut and rinsed with distilled water immediately before the experiment began. The woody cylinder was 3 cm. long. It was supported horizontally, and a tongue of bibulous paper was applied to the outer surface of the wood and hung down vertically into a little phial which received the drops of transmitted water. The time required for five drops to fall from the tongue was noted by means of a stop-watch, and the weight of these five drops determined by weighing the phial. The mean of three such observations is given below (Table V) for each pressure.

These results confirm the received view that the amount transmitted varies directly with the pressure. The slight bending over of the line joining the observations at the high pressures is amply explained by the gradual clogging which takes place despite all precautions; for the experiments giving these results were made with the same piece of wood after those at lower pressures had been carried out.

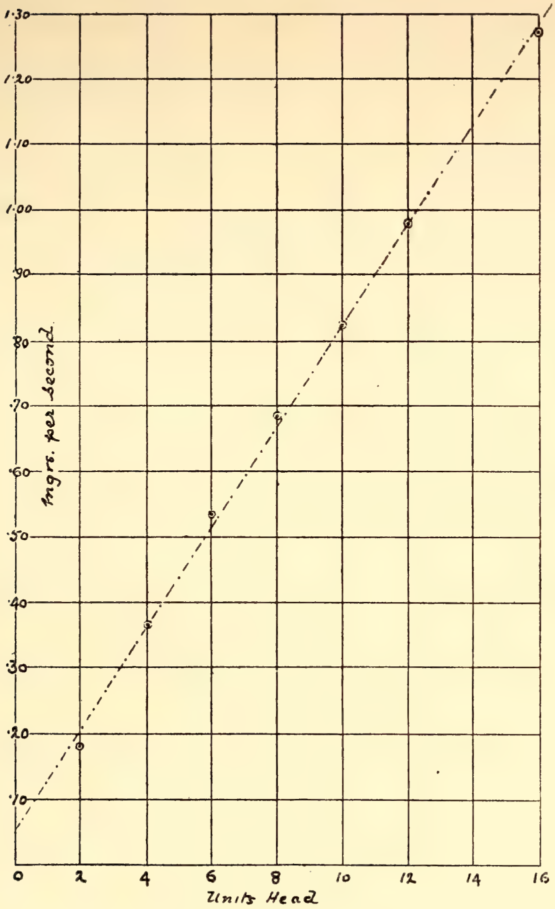

FIG. 3.

Table V.

Head.	Milligrammes transmitted.	Time.	Milligrammes per second.
centimetres.		seconds.	
6	44·0	246	0·182
12	50·0	137	0·365
18	53·0	99	0·535
24	50·0	73	0·685
30	52·0	63	0·825
36	58·0	60	0·966
48	59·5	47	1·266

The proportionality of head to flow may be indirectly investigated in another manner. A straight branch fixed in a horizontal position is connected to a large vessel of distilled water. Precautions are taken to clean the connections and the freshly cut inner end of the branch. A tongue of bibulous paper is applied to the outer end to draw off the transmitted water. The head once adjusted is kept constant; but after each determination the branch is shortened. Fig. 4 records such a series of experiments. The head throughout these was 100 cm. The initial length was 25 cm. At that length the flow was 1·18 mg. per second. Five centimetres were then cut off the outer end and the flow rose to 1·66 mg. per second. A shortening to 15 cm. increased the flow to 2·33 mg. When the branch was 10 cm. and 5 cm. long, the flow was 3·79 mg. and 6·70 mg. respectively. The curve plotted in fig. 4

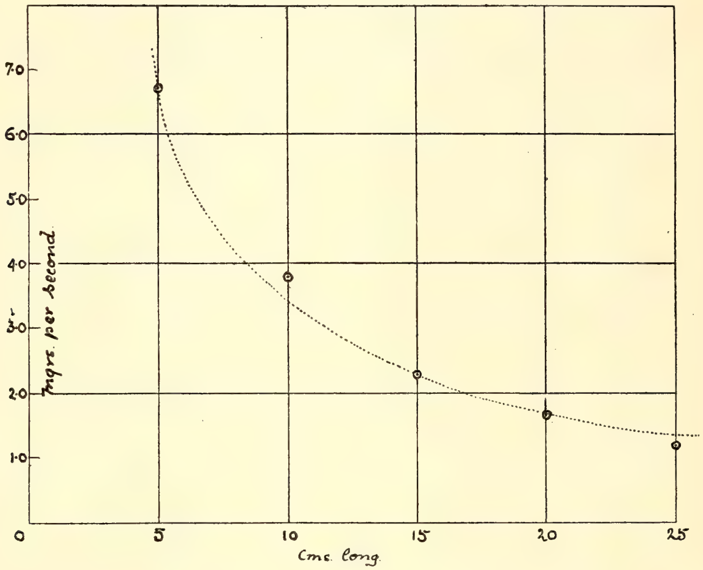

Fig. 4.

is a rectangular hyperbola in which $M = K/l$; M being the number of milligrammes transmitted per second, l = length of wood, and K = average value of the product Ml observed. The observations, it may be seen, approximate fairly closely to this curve. When the weights of transmitted water are plotted against units head the curve shown in fig. 5 is obtained. Here the proportionality of flow to head, or, rather, the inverse proportionality of flow to length, is immediately apparent. Up to 10 units the curve is almost a straight line. The bending over which occurs after that point is to be attributed to the clogging, which is practically unavoidable when the flow is rapid.

In conclusion, it seems to me that Ewart's objections to the cohesion-theory, based as they are on an excessive estimate of the velocity of the transpiration current and of the resistance to this current in the conduits,

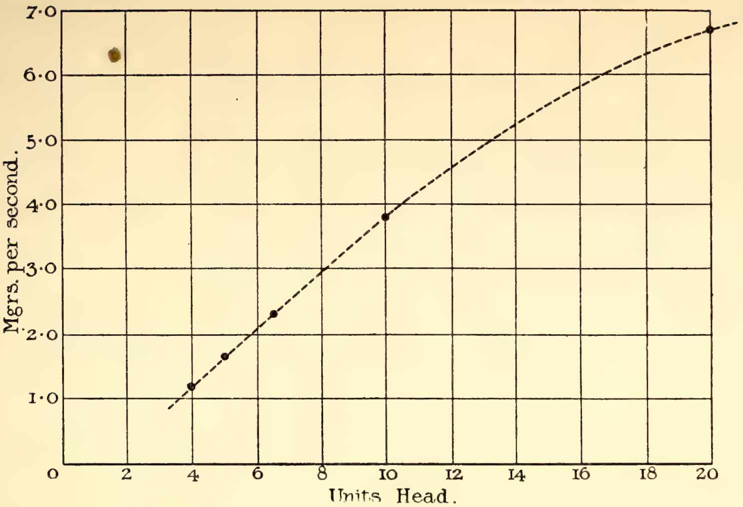

FIG. 5.

fall to the ground. With regard to the soundness of the cohesion-theory itself, apart from the weighty evidence which has elsewhere been adduced in its favour,* the fact that other theories, both old and new, have to assume properties for the water-ways of plants which are either in the highest degree improbable according to received scientific views, or are even directly negatived by experiment, seems to support the theory by a process of exclusion.

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* Dixon and Joly, "Ascent of Sap," 'Phil. Trans.,' B, 1895; Dixon, "Physics of the Transpiration Current," 'Notes from the Botanical School, Trinity College, Dublin,' May, 1897.

Nitrification of Sewage.

By George Reid, M.D., D.P.H.

(Communicated by Professor Gotch, F.R.S. Received May 7,—Read November 1, 1906.)

My special object in this paper is to direct attention to certain observations I have recently made bearing upon depth of filters and grade of particles as affecting both the quality of the work done and the capital outlay involved.

To effect the necessary changes it is essential that the sewage in a fine film shall be brought into intimate contact with the nitrifying organisms in the presence of an adequate supply of oxygen. It follows, therefore, that the larger the number of organisms employed, so long as they are maintained in a healthy active condition, the greater the amount and the better the quality of the work done. How then should a filter bed be constructed, so that, within a given cubic space, it shall afford the largest possible surface for bacterial growth under healthy conditions? Clearly, I suggest, by reducing the particles composing the filter to the smallest size which is found to be compatible with free aeration.

Compare, from the point of view of effective working area, a filter formed of 2-inch cubes—a grade in common use at present—with one formed of $\frac{1}{8}$-inch cubes, and it will be found that, bulk for bulk, the area in the latter case is 16 times greater than in the former, but taking into account the relatively larger space occupied by the smaller particles, the actual gain is as 1 to 14·7.

The question is, then, to what extent in actual practice is it possible to reduce the size of the filter particles? Some four years ago I had an opportunity of conducting an experiment on a large and practical scale at Hanley with a plant which complied with all the conditions which previous experience had shown me to be essential, including, for the first time I believe, perfect and uniform distribution of the sewage on to the filter by means of a power-driven apparatus.

Shortly, this plant, which dealt with 500,000 gallons per day (1,000,000 gallons per acre), consisted of:—

1. A detritus tank and strainer of the usual type.

2. Three subsidence tanks having a total capacity equal to one-eighth the daily dry weather flow.

3. An open septic tank with a capacity of seven-eighths the daily flow

(giving, with the subsidence tanks, a capacity for quiescence equal to a day's flow).

4. Two quarter-acre "percolating" filters 4 feet 9 inches deep, the one circular and the other rectangular, and each having a different type of power-driven distributor. I may here mention that I have long since discarded the "contact" method of working bacteria beds in favour of what is termed "percolating" filtration.

The filtering medium in this case consisted of broken "saggars," the discarded hard-burned clay vessels in which china is packed for firing, and the filters were constructed in four separate sections, each having different graded material. This material ranged (in separate sections) from 1½ inch to ⅛ inch. I have elsewhere given a full description of the working of this plant during a period of about eighteen months, and the results proved that such filters, working at the rate of 200 gallons per square yard per 24 hours (1,000,000 gallons per acre) continuously, produce a high-class effluent, the best results being obtained from the section of the filter composed of the finest grade particles (⅛ inch).

So much for the preliminary work which led up to the present investigations, the results of which seem to me to be of scientific interest and of practical importance.

It would seem to be established that the nitrification of hydrolysed sewage takes place in stages, the nitrous change being effected by one set of organisms as a preliminary to the nitric change brought about by another set, and the theory is that the two sets of organisms are located in different layers of the filter. With the view of verifying this, and, at the same time, ascertaining the degree of purification effected at different depths, I determined to tap the filter which had been used for the purpose of the experiment already referred to, in such a manner that samples might be collected at different levels, and I selected the finest grade section for this purpose (⅛-inch particles). Accordingly, four longitudinal shallow trays, with perforated covers, were placed in the body of the filter, the first at 1 foot from the surface, the second and third at 1-foot intervals downwards, and the lowest 18 inches below the third one, pipes being carried from the various collecting trays through the wall of the filter, to allow of the collection of the respective samples. In order that the results from the lower depths might not be affected by the presence of the trays above, the trays were placed obliquely from above downwards, so that no tray had another in the vertical line above it. As the filter at this time had been constantly at work for two and a-half years, in replacing the filtering material which had been disturbed in order to introduce the trays care was taken to ensure as far as possible that each portion was placed in the position it had previously occupied in the depth of the filter, and seven days were allowed to elapse for recovery before collecting the first samples.

As regards analytical methods, the free ammonia was determined in the usual way by distillation and Nesslerising, and the albuminoid ammonia by Wanklyn's process, the quantities taken respectively being—sewage, detritus tank, and septic tank, 50 c.c., filter effluents, 500 c.c., the former being made up to 500 c.c. by ammonia-free water. The oxygen absorbed was determined by Tidy's process, the nitrous nitrogen by Griess' method, and the nitric nitrogen by Sprengel's method.

In order to discount possible subsequent changes from the lower to the higher oxide, and *vice versâ,* the tests for nitrous and nitric nitrogen were made at the works immediately on the collection of the samples, and again in the laboratory the following day, when the complete analyses were made.

The following figures represent the mean results of a series of analyses, the individual figures of which are given in the detailed tables attached. The samples were collected at intervals extending over a period of about six months, the filters being steadily worked at the previous rate, namely, 200 gallons per superficial yard per 24 hours, and the delivery being uniform and continuous day and night.

In the first place, before commenting generally on the conclusions which may be drawn from the results as a whole, I propose to refer shortly to each analytical record separately:—

1. With regard to the suspended solids it will be noticed that a reduction of 73 per cent. is effected in the detritus tanks, and that a further reduction of 15 per cent. takes place in the septic tank, making a total reduction of 88 per cent., and resulting in an effluent being passed on to the filter containing 7·6 parts per 100,000, exactly one-half of which is mineral matter. This suspended matter, it will be seen, is practically all retained in the top layer of the filter, where the organic portion is liquefied, in all probability, by aerobic organisms. The mineral matter, however, must remain in the filter, and in time, no doubt, it will be found necessary to remove the filtering medium to a depth of a few inches for the purpose of washing it, but so far, after over three years' constant working, no such necessity has arisen. As a matter of fact, if the total mineral suspended solids passing on to the filter during the three years were deposited in a uniform layer over the whole surface, the depth of the coating would be less than $1\frac{1}{4}$ inch.

2. The reduction of the free ammonia at a depth of 1 foot is remarkable, especially considering the fact that the change has been effected in about

Sample.	No. of records.	Total solids.	Solids in suspension.	Solids in suspension, organic.	Solids in suspension, mineral.	Chlorine.	Free ammonia.	Albuminoid ammonia.	Oxygen absorbed in 4 hours at 80° F.	Oxygen absorbed in 3 mins. before incubation at 80° F.	Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	Nitric nitrogen on day of collection.	Nitric nitrogen, day after collection.	Nitrous nitrogen on day of collection.	Nitrous nitrogen, day after collection.	Depth of column necessary to obscure test lines.
																inches.
Sewage	18	170 ·9	63 ·5	28 ·5	34 ·9	11 ·0	2 ·154	0 ·972	5 ·019	1 ·862	2 ·176	0 ·02	0 ·10	0 ·029	0 ·029	0 ·5
Detritus tank ...	13	118 ·1	17 ·0	6 ·8	10 ·1	10 ·0	1 ·643	0 ·486	2 ·726	0 ·975	1 ·095	0 ·02	0 ·09	0 ·014	0 ·022	1 ·6
Septic tank	16	107 ·8	7 ·6	3 ·8	3 ·8	9 ·9	1 ·716	0 ·340	2 ·184	0·836	1 ·571	Nil	0 ·09	Nil	Nil	1 ·5
Filter, 1 ft.......	16	101 ·5	0 ·25	0 ·16	0 ·08	9 ·4	0 ·036	0 ·052	0 ·328	0 ·093	0 ·067	1 ·64	2 ·07	0 ·003	0 ·003	Over 24
„ 2 „	16	101 ·1	0 ·09	0 ·005	0 ·003	9 ·5	0 ·020	0 ·037	0 ·286	0 ·077	0 ·060	1 ·82	1 ·99	0 ·011	0 ·007	„
„ 3 „	16	101 ·8	0 ·14	0 ·06	0 ·08	9 ·4	0 ·009	0 ·031	0 ·244	0 ·060	0 ·052	1 ·75	1 ·85	0 ·005	0 ·008	„
„ 4·5 „	16	103 ·5	—	—	—	9 ·5	0 ·043	0 ·027	0 ·259	0 ·070	0 ·039	1 ·70	1 ·99	0 ·005	0 ·002	„

12 minutes—the time occupied by the sewage in passing downward through the first foot of filter. In my experience it is not unusual to find the free ammonia figure reduced almost to an equal extent in effluents from fine-grade filters, but, hitherto, I had no conception that the change was brought about by so shallow a depth of filter.

I may here mention that the rate of travel downwards of the sewage through the filter was found, as the result of several observations, to vary in accordance with the depth as follows:—

From surface	to 1 ft.	12 mins.
" 1 ft.	" 2 "	12 "
" 2 "	" 3 "	6 "
" 3 "	" 4 ft. 6 in.	5 "
	Total	35 "

This is a slow rate of travel compared with the rate in the case of large particle filters, which, in my experience, in some cases allow the sewage to pass through the entire depth of from 4 to 5 feet in about four minutes.

The rate of travel was ascertained by a series of observations as follows:—The delivery on the filter was stopped until the discharge pipes from the respective trays showed unmistakable signs of diminished flow, when it was started again, the period which elapsed before the discharge from the pipes was restored being taken as the time occupied in the passage downward of the sewage to the different depths.

The free ammonia figure presents one other interesting feature. It will be noticed that the progressive reduction which takes place during the passage downwards through the first 3 feet is suddenly interrupted, a considerable increase in the amount being recorded at a depth of 4 feet 6 inches. Accidental error in analysis does not explain this, for it will be seen on referring to the detailed tables that this increase is invariably recorded, and, as a matter of fact, I verified the accuracy of the results by a second analysis in the case of the first few samples until it became quite obvious that no such explanation of the occurrence was feasible. This phenomenon will be referred to later in dealing with the general arguments and conclusions.

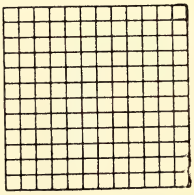
3. As regards the albuminoid ammonia figure, it will be seen that a highly satisfactory reduction is effected by filtration through 1 foot only. In fact, this figure and the oxygen absorbed and nitric nitrogen figures considered together indicate a very high degree of purification, which precludes the possibility of subsequent putrefactive change.

4. It will be seen that the reduction in the oxygen absorbed is, stage by

stage, proportionate to the albuminoid ammonia reduction, and the rapidity of the change in this case also is equally marked.

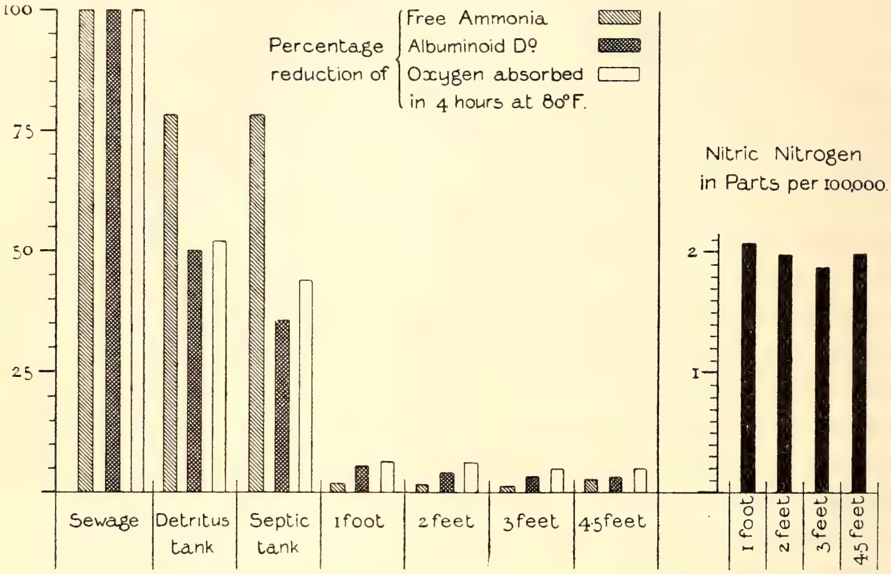
5. With reference to the oxidised nitrogen the figures are very startling, for not only are they indicative of extremely healthy and active biological conditions, but, considered in relation to the other figures, they also demonstrate conclusively, in the case of the sewage in question at any rate, that the work of purification is practically completed within a few inches of the surface of the filter. Within 12 minutes a foul and offensive liquid is rendered not only clear and non-odorous, but also absolutely stable as regards putrescible qualities, as is proved by the oxygen absorbed figures before and after incubation. I must say that this result surprised me very much. Theoretically, as I have said, one expected to find that the process was a more gradual and progressive one, nitrites being formed chiefly in the superficial layers of the filter and nitrates in the deeper layers, but this did not prove to be the case. At the same time I do not suggest that the change is not gradational, but rather that the two sets of organisms are at work side by side, and in this case the absence of more than a trace of nitrous acid in the 1-foot effluent, although the tests were applied at the moment of collection, may be accounted for by the preliminary stage of the oxidation process being an extremely evanescent one owing to the highly efficient working conditions.

6. The column headed "depth of column necessary to obscure test lines" requires explanation. It is a standard opacity test which I adopted some years ago for Staffordshire, and which has since been adopted by some other river authorities. The lines referred to are engraved on a white porcelain disc, which is attached by means of a socket and rubber washer to a 2-foot glass tube, graduated in half-inches. The exact mesh and depth of lines are as shown in the drawing, and in fixing the scale as regards mesh and length of tube, in the first instance, I was guided by my experience at the time as to the opacity of well-clarified effluents. I have since found, however, that the clarification effected by fine-grade filters and efficient distribution is so perfect that the lines are not usually obscured by a depth of 24 inches, and this was the case in the present instance. It will be noticed that while the mean depths of fluid necessary to obscure the lines in the case of the crude sewage and the detritus and septic tank effluents were 0·5, 1·6, and 1·5 inches respectively, the filter effluents, even those from the 1-foot tray, were so highly clarified that the full 24-inch column did not suffice to obscure the lines. It is true that the opacity test in itself is not a reliable index of purity;



at the same time, such a record, considered in conjunction with that of the suspended solids, is useful for comparative purposes.

For convenient reference, I have shown in the form of a diagram the percentage reduction in free ammonia, albuminoid ammonia, and oxygen absorbed at different stages, the figures for the sewage being taken at 100, and also the actual nitric nitrogen figures in parts per 100,000.



So much, then, for the detailed results of the working of this plant. Whether what has been proved to be possible in this case will be found to be equally possible in other cases remains to be seen, and if the answer be in the affirmative, it is unnecessary to point out that the resulting economy in the construction of filters would be very material. Personally I am extremely optimistic regarding future possibilities in this direction, and only the other day I had an opportunity of practically applying this recent experience with, so far, most gratifying results, in the case of a stronger sewage and one which contains a considerable amount of brewery waste.

In this case the authority had some years ago constructed works which provided for the nitrification of the sewage by means of "contact" beds, and, being lately threatened with proceedings on account of pollution, on the advice of their engineer an experimental plant was constructed, with the view of determining whether better results could be obtained by means of "percolating" filters. Having worked these filters for about seven months, the engineer came to the conclusion that he might safely recommend the adoption of a scheme on similar lines, but with 5-ft. 6-in. filters, to dispose of the whole of the sewage of the town. Before assenting to this proposal, however, the authority decided to seek further advice, and this led to my being consulted.

Shortly, I may state that the experimental filter in this case had a mean depth of 4 feet, and was formed of clinker broken to sizes varying from $\frac{3}{4}$ to $\frac{1}{2}$ inch, the sewage, after having undergone preliminary treatment, the nature of which need not here be described, being distributed on to the filter by means of a series of fixed sprays. The results obtained were fairly satisfactory as regards nitrification, but the organic matter remaining unconverted was considerable, and the effluent, so far as appearance went, was a poor one, owing to the amount of suspended matter it contained.

In order to determine whether and to what extent the imperfect results were attributable to the comparatively large-sized medium composing the filter, and with the view of ascertaining whether, in the case of the sewage in question, a shallower filter would answer the purpose, I suggested that a section of the filter should be partially reconstructed by removing the top medium to a depth of 12 inches and replacing it by granite chippings broken to $\frac{1}{8}$ inch, and also that a collecting tray should be introduced at a depth of 3 feet in the reconstructed section. This alteration was made, and we thus had the means of comparing the results obtained by the altered section at depths of 3 feet and 4 feet 6 inches with those from the undisturbed section at a depth of 4 feet 6 inches under identical conditions.

Having resumed the working of the filter, at a rate of flow of 200 gallons per square yard per day, three weeks were allowed to elapse for ripening before the first set of samples were collected, and up to the time of writing this paper the experiment, under the altered conditions, had been in operation for four months.

So far I have had an opportunity of analysing two sets of samples from the two sections of the filter, and the following are the means of the more significant results obtained:—

	Parts per 100,000.			
	Septic effluent.	Unaltered section of filter at 4 ft. 6 in. deep.	Altered section of filter at 4 ft. 6 in. deep.	Altered section of filter at 3 ft. deep.
Solids in suspension	9·10	2·10	1·15	1·00
Free ammonia	1·067	0·710	0·250	0·067
Albuminoid ammonia	0·558	0·154	0·107	0·044
Oxygen absorbed in 4 hours at 80° F.	3·157	0·818	0·680	0·388
Nitric nitrogen	Nil	1·04	1·16	2·58

It will be seen that the improvement effected by apparently so trivial an alteration is remarkable. The improved quality of the effluents from the altered section of the filter at a depth of 3 feet compared with those from the same section and the unaltered section at a depth of 4 feet 6 inches is most striking.

With reference to the work done at different depths in filters, Scott-Moncrieff has added very much to our knowledge of the subject by his well-known experiments: on a small scale at Ashtead some eight years ago, and, later, on a larger scale, at Caterham Barracks. The filters were built up of nine trays, each 7 inches deep, with perforated bottoms, and supported vertically over one another with about 3-inch interspaces. The depth of the filtering medium in each tray was 6 inches, giving a total effective depth of 4 feet 6 inches. The medium consisted of coke, broken to pass through a ring 1 inch in diameter, and the rate of delivery was about 200 gallons per square yard (1,000,000 gallons per acre).

The object Scott-Moncrieff had in view was a two-fold one: first, to secure the freest possible aeration of the filter; and, secondly, to separate, as far as possible, the various stages of the process by providing facilities for the differentiation of the colonies of nitrifying organisms. Incidentally the arrangement also allowed of the collecting of samples of effluents for analysis from different depths, for comparative purposes as regards the oxidising changes.

Without going into detail, I may mention that while the nitrification effected by the tray filters was exceptionally high, the changes, notwithstanding the free aeration which the arrangement provided for, did not take place so rapidly, nor at so shallow a depth, as in the case of the Hanley experiments. The nitrous change was more marked, and was continued to a greater depth, and the reduction in the free ammonia, as well as in the oxygen absorbed, was far more gradual. It is true that the sewage was a much stronger one, but, making allowance for this, the work done per foot deep of filtering medium was relatively considerably less than in the case of Hanley. I feel pretty confident that had a much finer filter medium been used by Scott-Moncrieff the high oxidising changes would have been effected at a shallower depth, and practically the whole of the suspended solids would have been liquefied and nitrified within the filter.

Dr. Harriette Chick, in a paper published in the 'Proceedings of the Royal Society,' 1906, B, vol. 77, gives an account of certain laboratory experiments she conducted in 1901, and again in 1903, having reference to the nitrification of sewage. Three cylindrical filters were employed, each 4¾ inches in diameter, and approximately 1½, 3¼, and 6½ feet in depth respectively. The filtering material used was coke of practically the same grade as that used at Hanley, and the sewage was delivered on to the filters at the rate of 300 gallons per 24 hours by means of a syphonic discharge on to a perforated distributing disc at intervals of 38 minutes. The sewage dealt with was artificially prepared by diluting liquid cow manure with water, the dilution in the first experiment being 1 in 20 and in the second 1 in 50 to 1 in 100. The suspended solids were removed by rough filtration through glass wool.

In this case, the filters being new, it was possible to determine the stages in the ripening process, and it was found that while a marked amount of general oxidation was apparent in the effluents from all the filters in two weeks, no trace of oxidised nitrogen appeared until 10 days later, and until then the ammonia in the effluents was the same as in the sewage. The first trace of oxidised nitrogen in the effluents appeared in the form of nitrous nitrogen on the following dates, all three filters having been started on February 20th:—

Deep filter on 18th March.
Medium „ 21st March.
Shallow „ 28th March.

For some time the formation of nitrous nitrogen continued in increasing amount, and later, when nitric nitrogen made its appearance, the nitrous nitrogen gradually diminished until ultimately the effluents from the medium and deep filter showed no trace of it. For some reason it would appear that the effluents from the shallowest filter ceased to be recorded before this period.

Regarding the absence of nitrous nitrogen in the effluents after the filters had matured, the results accord with those at Hanley. In one other respect, namely, the reduction of general oxidisable matter, the results also agree with those at Hanley in the fact that practically the reduction was as marked in the shallow as in the deep effluents.

As regards the degree of nitrification at different depths, Dr. Chick's results do not quite compare with those of Hanley. It must be remembered, however, that the conditions differed widely. The volume of sewage dealt with in the former case was relatively greater, and its ammoniacal strength was very much higher. Also the intervals of delivery on to the filter were much longer, namely, 38 minutes compared with 5 minutes in the case of Hanley, and in addition all the disturbing influences incidental to a laboratory experiment of this character have to be taken into account, such as the effect of peripheral capillarity upon the uniformity of the downward flow in a filter of such small diameter. At the same time, the nitrification effected at the medium depth was very considerable, and it would have been interesting to have had another record between that and the 6-ft. 6-in. depth.

It may now be convenient to sum up the arguments which may be adduced, and the conclusions which may be drawn, from the recent observations at Hanley. In the first place, two important factors undoubtedly contributed in no small degree to the high quality of the work done: (1) the small amount of suspended matter in the effluent applied to the filter; and (2) the very efficient means of distribution provided.

The oxidation of organic matter already in solution is a comparatively simple process, but to bring about the liquefaction of suspended organic solids takes longer time, hence the importance of providing, as far as possible, for their removal by mechanical subsidence or other methods, and their liquefaction by anaerobic organisms as a preliminary process. When septic tank treatment was first introduced, its advocates, in their enthusiasm, predicted that the difficult question of sludge disposal would thus be solved, but experience does not quite corroborate this prediction. In the case in point I attribute the satisfactory reduction of suspended solids in the septic tank effluent to the large capacity of the detritus tanks. These were divided into three, two of which were always in use, while the third was emptied for cleansing weekly, and so on in rotation, the whole three being brought into use during rain periods.

As regards the distribution of the sewage on the filter, it is obvious that uniformity is all-important, otherwise certain sections might be greatly overtaxed, while other sections were working much below their capacity. The distribution in this case was so perfect that frequent tests failed to establish any appreciable difference between one square yard of filter and another.

Now, as regards the rapidity of the oxidising changes, and the remarkable purity of the effluent after filtration through 12 inches only. The three essential factors in the final changes are time, air, and organisms, and, given a sufficiency of air, the greater the number of organisms present the larger the amount of work done, provided the organic matter both in solution and suspension is brought into intimate contact with the organisms. The factor which governs the bacterial population is the area available for growth, and this may be increased by two methods, either by enlarging the cubic capacity of the filter or by subdividing the filtering medium. In the case in point, the latter was the expedient adopted, and the subdivision was carried as far as it was thought possible to carry it without preventing the superficial penetration of the suspended solids into the interstices. In view of the results, it is needless to discuss whether the reduction in the size of particles resulted in an inadequate air supply.

The relative amount of carbonic acid in the air of the filter at different depths also shows the highly active oxidising changes which take place in the superficial layers. A series of samples of air aspirated from different depths, by means of iron tubes driven vertically into the body of the filter while in continuous use, yielded the following mean results:

Carbonic Acid in parts per 1000.

1 ft.	2 ft.	3 ft.	4 ft.
19·5	21·5	20·0	20·0

An interesting fact bearing upon the aeration of the filter was incidentally made apparent by the method at first adopted of collecting the air. The distributor passing backwards and forwards over the filter was found to interfere considerably with the collection of the samples, and, to obviate this, the apparatus was periodically stopped for varying periods during the operation. Owing, however, to extraordinary discrepancies in the results thus obtained, measures were taken to overcome this difficulty and enable the air to be collected without interrupting the regular flow of sewage on to the filter. The results, of which the figures just given are the means, were then found to be remarkably uniform, whereas in the case of the earlier samples the carbonic acid varied in amount according to the intervals which elapsed between the stopping of the distributor and the collection, short though these intervals were, from 2·8 to 26·1, showing how free was the current of air through the filter.

If, then, by using fine-grade particles the depth of filter may be greatly reduced, the resulting economy would dictate such a course, but there is another important consideration which, other things being equal, tells in favour of shallow filters from the point of view of aeration. The air travels through a filter from above downwards, the direction of the current probably being mainly due to the percolation downwards of the sewage, and its more rapid flow along the effluent drains. The air, therefore, as it passes downwards, carries with it the products of the combustion which has taken place above, and thus has an asphyxiating effect upon the organisms below, and it is possible that even anaerobic fermentation may be revived in the bottom layers. The sudden increase in the free ammonia figure, noted in the case of the effluent from the lowest tray, may possibly be accounted for in this way, because the albuminoid ammonia figure does not represent the total organic nitrogen present, therefore there is an unrecorded margin of nitrogenous organic matter available for the revival of the ammonia change should this explanation of the phenomenon be the correct one.

Be this as it may, however, the phenomenon does not appear to be accounted for by fouling of the deeper strata. At the end of the observations the filter was opened and carefully examined throughout its depth, when it was found that the dark discoloration from deposit was confined to the surface, as was evident from the untarnished appearance of the light-coloured filtering medium below the top 14 to 18 inches. Also, the relative amount of organic solids in the interstices at different depths, ascertained by drying and igniting 10 grammes of the filter particles in each case, supports this contention, as the following figures show:—

Percentage Loss on Ignition of Filter Particles at Different Depths.

6 in.	1 ft.	2 ft.	3 ft.	4 ft.
3·25	0·99	0·65	0·53	0·53

I may mention that on ignition there was practically no smell, except in the case of the samples collected at the 6-inch and 1-foot depths, and that in the case of these only an odour such as that of burning soil could be detected.

Apart from all theory, however, the fact has been established beyond all doubt that, in the case of the sewage in question at any rate, the lower or 3 feet of filter medium is absolutely unnecessary, and, so far as the cost of construction of the filter is concerned, the expenditure might be reduced by about one-half. Again from the point of view of cost, another important consideration comes in. It frequently happens that the absence of 2 or 3 feet of available fall is the determining factor between a gravitation and pumping scheme, and in this respect the reduced depth of filter might lead to further economy, not only in capital outlay, but in maintenance charges.

Now, the question may be asked whether this experience acquired at Hanley may be applied in other cases where the sewage may be of a stronger character? I am not at present in a position to give a positive answer to that question, but if, as is probable, such should prove not to be the case, the observations I have recorded clearly point to the conclusion that the extra filtering capacity should be provided for by increasing the area rather than the depth of the beds. The three factors in the nitrifying process being a given time, a given volume of air, and a given bacterial population, all these would be supplemented by extending the area in accordance with the combustion which has to be effected, and thereby diminishing the delivery per square yard of filter. Of course, I do not suggest that the depth of filters may be reduced to 1 foot, because we must allow for the effluent drains, and the few inches of large material immediately on the top of them, but I do suggest the practicability, if a fine medium is used, of reducing the total depth to, say, 2 feet 6 inches.

The tables appended give the details of the analyses and other determinations made during the investigations which have been described.

Crude Sewage. Parts per 100,000.

1905.	July 6.	July 13.	July 20.	Aug. 24.	Aug. 31.	Sept. 7.	Sept. 15.	Sept. 21.	Oct. 9.	Oct. 13.	Oct. 19.	Oct. 26.	Nov. 2.	Nov. 10.	Nov. 17.	Dec. 7.	Dec. 14.	Dec. 21.
Total solids	162·8	—	189·0	142·4	167·0	156·4	147·5	—	239·2	155·5	187·9	199·3	156·0	172·0	162·5	162·4	167·9	166·8
Solids in suspension ...	—	—	—	46·0	66·2	43·8	43·0	—	133·0	53·2	74·8	65·8	47·7	78·5	62·1	51·7	63·2	60·5
,, organic	—	—	—	19·0	33·0	18·5	22·0	—	35·8	23·8	37·8	34·2	20·3	41·8	30·8	23·4	30·9	29·0
,, mineral	—	—	—	27·0	33·2	25·3	21·0	—	97·2	29·4	37·0	31·6	27·4	36·6	31·2	28·3	32·3	31·5
Chlorine	9·2	11·2	10·6	13·6	12·4	11·8	10·8	10·2	11·2	12·4	11·8	11·2	9·6	12·6	8·8	10·8	11·4	9·4
Free ammonia	1·516	2·376	3·288	1·666	1·668	1·656	2·220	2·880	2·544	2·180	2·240	2·350	1·904	3·148	2·120	1·674	1·600	1·750
Albuminoid ammonia	0·824	0·828	1·084	1·030	0·844	0·710	0·850	1·068	1·704	0·592	0·916	1·532	0·724	0·846	0·890	0·880	1·086	1·096
Oxygen absorbed in 4 hours at 80° F.	3·400	4·840	5·564	4·736	4·034	3·900	4·834	4·867	6·667	4·234	5·813	5·300	4·500	5·157	4·657	6·20	5·800	5·844
Oxygen absorbed in 3 mins. before incubation at 80° F.	—	—	1·626	1·613	1·700	1·667	1·734	2·220	2·434	1·800	2·188	2·00	1·234	1·813	1·782	1·970	1·800	2·219
Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	—	—	—	—	—	—	—	2·867	2·034	1·634	2·000	2·967	1·667	1·875	1·407	1·700	2·634	3·157
Nitric nitrogen on day of collection	—	—	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0·25	—	0·10
Nitric nitrogen day after collection	Nil	Nil	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	0·95	0·47	0·39	0·10
Nitrous nitrogen on day of collection	—	0·022	0·022	0·04	0·03	0·018	0·007	,,	0·09	0·09	0·02	0·03	0·018	0·029	0·014	0·011	0·025	Nil
Nitrous nitrogen day after collection	—	Nil	Nil	Nil	Nil	Nil	0·007	,,	0·114	0·129	0·051	0·007	0·062	0·04	0·014	0·03	0·06	,,
Column necessary to obscure test lines (inches)	0·5	0·5	0·75	0·75	0·75	0·5	1·0	0·5	0·25	0·5	0·5	0·5	0·5	0·5	0·5	0·75	0·5	0·25

Detritus Tank Effluent. Parts per 100,000.

1905.	Aug. 24.	Aug. 31.	Sept. 7.	Oct. 9.	Oct. 13.	Oct. 19.	Oct. 26.	Nov. 2.	Nov. 10.	Nov. 17.	Dec. 7.	Dec. 14.	Dec. 21.
Total solids	123·0	131·5	141·9	122·4	86·1	116·4	121·8	107·8	104·1	107·2	141·1	116·9	115·7
Solids in suspension	26·0	22·6	23·1	17·1	19·2	19·6	16·3	13·4	10·68	10·6	8·2	17·8	16·9
,, organic	10·6	8·1	8·1	11·3	9·2	8·7	1·9	2·2	4·64	5·3	1·8	8·8	8·8
,, mineral	15·4	14·5	15·0	5·8	10·0	10·9	14·4	11·2	6·04	5·3	6·4	9·0	8·1
Chlorine	15·8	13·4	11·8	9·6	8·0	9·4	9·0	8·6	7·6	9·0	9·2	9·8	9·2
Free ammonia	2·176	1·742	1·990	2·100	1·692	2·000	1·368	1·476	0·960	1·428	0·816	1·600	2·018
Albuminoid ammonia	0·802	0·556	0·846	0·592	0·330	0·444	0·218	0·290	0·245	0·318	0·348	0·604	0·726
Oxygen absorbed in 4 hours at 80° F.	3·484	3·434	3·700	2·700	2·500	2·344	2·400	1·467	1·719	1·938	2·334	3·634	3·782
Oxygen absorbed in 3 mins. before incubation at 80° F.	1·355	1·267	1·434	0·867	1·000	0·813	0·867	0·534	0·688	0·625	1·067	0·867	1·294
Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	—	—	—	1·000	1·000	0·969	1·000	1·500	0·594	0·688	1·134	1·634	1·438
Nitric nitrogen on day of collection	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0·25	—	0·09
,, ,, day after collection	,,	,,	,,	,,	,,	,,	,,	,,	,,	0·42	0·25	0·42	0·09
Nitrous nitrogen on day of collection	0·03	0·029	0·01	,,	,,	0·01	0·02	0·007	0·01	0·01	0·007	0·02	0·02
,, ,, day after collection	Nil	Nil	Nil	,,	0·059	Nil	0·077	0·040	0·062	0·011	0·02	0·04	0·014
Column necessary to obscure test lines (inches)	1·25	1·25	1·0	1·25	1·5	1·5	1·75	1·5	2·5	2·75	2·0	1·5	1·25

Septic Tank Effluent. Parts per 100,000.

1905.	July 13.	July 20.	Aug. 31.	Sept. 7.	Sept. 15.	Sept. 21.	Oct. 9.	Oct. 13.	Oct. 19.	Oct. 26.	Nov. 2.	Nov. 10.	Nov. 17.	Dec. 7.	Dec. 14.	Dec. 21.
Total solids	—	126·7	94·2	102·5	100·5	—	99·4	98·1	108·4	117·5	102·0	108·3	115·7	113·6	110·8	112·2
Solids in suspension	—	—	2·6	5·4	12·0	—	2·9	7·6	10·0	7·4	9·6	10·8	6·7	6·3	8·8	8·6
,, organic	—	—	0·7	0·3	6·6	—	1·3	5·7	5·5	2·0	3·8	7·0	3·5	3·7	5·3	4·8
,, mineral	—	—	1·9	5·1	5·4	—	1·6	1·9	4·5	5·4	5·8	3·8	3·2	2·6	3·5	3·8
Chlorine	9·8	10·8	8·6	10·0	9·2	9·4	10·6	10·0	9·8	10·6	11·2	9·6	8·8	10·2	9·8	10·0
Free ammonia	1·684	2·592	1·214	1·706	1·538	2·268	1·584	2·076	1·036	1·882	2·020	1·614	1·650	1·090	1·492	2·024
Albuminoid ammonia	0·208	0·296	0·224	0·394	0·358	0·320	0·286	0·238	0·265	0·300	0·290	0·374	0·354	0·336	0·476	0·734
Oxygen absorbed in 4 hours at 80° F.	1·968	2·188	1·834	2·267	1·080	2·667	1·734	1·967	1·813	2·470	2·367	2·157	1·625	2·434	2·667	3·719
Oxygen absorbed in 3 mins. before incubation at 80° F.	—	0·814	0·634	1·134	0·800	1·100	0·567	0·934	0·688	0·867	0·834	0·782	0·657	0·800	0·834	1·094
Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	—	—	—	—	—	1·834	0·967	1·234	1·313	2·667	1·334	1·625	1·407	1·900	0·967	2·032
Nitric nitrogen on day of collection	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
,, ,, day after collection	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	0·30	0·55	,,	,,	0·50
Nitrous nitrogen on day of collection	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	Nil	Nil	,,	,,	Nil
,, ,, day after collection	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
Column necessary to obscure test lines (inches)	1·5	1·25	1·75	1·25	1·5	1·25	2·0	1·5	2·0	1·75	1·5	1·5	2·5	1·5	1·5	1·0

Filter Effluent, 1 foot below Surface. Parts per 100,000.

1905.	July 13.	July 20.	Aug. 31.	Sept. 7.	Sept. 15.	Sept. 21.	Oct. 9.	Oct. 13.	Oct. 19.	Oct. 26.	Nov. 2.	Nov. 10.	Nov. 17.	Dec. 7.	Dec. 14.	Dec. 21.
Total solids	—	—	93·7	116·0	92·5	—	95·0	92·3	101·4	104·3	92·9	97·0	113·7	110·1	105·5	105·2
Solids in suspension	—	—	—	0·6	0·0	—	Nil	Nil	0·4	Nil	Nil	Nil	Nil	Nil	2·0	Nil
,, organic	—	—	—	0·2	0·0	—	,,	,,	0·2	,,	,,	,,	,,	,,	1·6	,,
,, mineral	—	—	—	0·4	0·0	—	,,	,,	0·2	,,	,,	,,	,,	,,	0·4	,,
Chlorine	9·6	11·0	8·4	9·4	9·0	9·6	10·0	9·8	9·0	10·2	8·6	9·2	8·8	9·0	9·0	10·0
Free ammonia	0·021	0·012	0·017	0·009	0·008	0·003	0·022	0·006	0·006	0·048	0·010	0·028	0·240	0·016	0·056	0·083
Albuminoid ammonia	0·038	0·035	0·057	0·027	0·034	0·041	0·039	0·053	0·027	0·033	0·029	0·048	0·069	0·086	0·158	0·061
Oxygen absorbed in 4 hours at 80° F.	0·336	0·276	0·268	0·280	0·336	0·308	0·308	0·308	0·276	0·336	0·268	0·352	0·312	0·456	0·440	0·388
Oxygen absorbed in 3 mins. before incubation at 80° F.	—	0·100	0·108	0·080	0·096	0·056	0·040	0·096	0·076	0·096	0·068	0·100	0·100	0·120	0·160	0·128
Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	—	—	—	—	—	0·056	0·056	0·040	0·064	0·080	0·068	0·100	0·028	0·108	0·136	0·000
Nitric nitrogen on day of collection	—	1·75	1·55	2·12	1·69	2·12	1·25	2·04	1·61	1·43	1·67	1·92	1·00	1·72	1·33	1·49
,, ,, day after collection	2·08	2·08	1·54	1·43	1·43	2·00	3·30	2·86	2·22	2·31	2·00	1·82	2·63	1·67	2·38	1·43
Nitrous nitrogen on day of collection	0·01	0·009	Nil	0·005	Nil	0·005	Nil	Nil	Nil	0·007	Nil	Nil	Nil	Nil	Nil	0·014
,, ,, day after collection	0·01	0·007	0·009	Nil	,,	Nil	,,	,,	,,	0·007	,,	,,	,,	,,	0·000	0·014
Column necessary to obscure test lines (inches)	14·0	22·0	16·0	Over 2′	Over 2′	Over 2′	Over 2′	19·0	Over 2′	Over 2′	Over 2′	2′	15·0	11·0	7·5	21·0

Filter Effluent, 2 feet below Surface. Parts per 100,000.

1905.	July 13.	July 20.	Aug. 31.	Sept. 7.	Sept. 15.	Sept. 21.	Oct. 9.	Oct. 13.	Oct. 19.	Oct. 26.	Nov. 2.	Nov. 10.	Nov. 17.	Dec. 7.	Dec. 14.	Dec. 21.
Total solids	—	119·5	96·3	89·2	93·5	—	96·8	92·5	101·8	104·9	94·6	93·3	114·4	110·2	104·1	105·2
Solids in suspension	—	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1·0	Nil
,, organic	—	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	0·6	,,
,, mineral	—	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	0·4	,,
Chlorine	9·8	11·0	8·6	9·0	9·4	9·6	10·3	9·8	9·6	10·2	8·8	9·2	8·8	9·6	9·0	9·8
Free ammonia	0·024	0·039	0·016	0·007	0·015	0·006	0·022	0·009	0·006	0·012	0·012	0·005	0·130	0·006	0·007	0·011
Albuminoid ammonia	0·045	0·039	0·038	0·023	0·024	0·036	0·028	0·034	0·019	0·026	0·025	0·046	0·045	0·062	0·072	0·043
Oxygen absorbed in 4 hours at 80° F.	0·416	0·328	0·280	0·216	0·256	0·240	0·280	0·280	0·216	0·268	0·216	0·288	0·264	0·348	0·360	0·328
Oxygen absorbed in 3 mins. before incubation at 80° F.	—	0·128	0·080	0·056	0·056	0·080	0·028	0·080	0·076	0·080	0·068	0·076	0·056	0·096	0·120	0·088
Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	—	—	—	—	—	0·040	0·040	0·040	0·052	0·068	0·056	0·064	0·052	0·096	0·096	0·064
Nitric nitrogen on day of collection	—	1·81	1·72	2·00	1·82	2·00	1·39	3·33	1·67	1·82	1·88	2·00	1·25	1·92	1·18	1·49
,, ,, day after collection	1·72	2·12	2·22	1·43	1·67	2·70	2·50	2·50	2·75	2·00	1·92	2·12	2·20	1·43	1·67	0·90
Nitrous nitrogen on day of collection	0·08	0·05	0·01	0·01	0·01	0·007	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
,, ,, day after collection	0·07	0·059	0·02	0·01	Nil	Nil	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
Column necessary to obscure test lines (inches)	5·0	4·5	13·0	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	14·0	10·5	Over 2·

Filter Effluent, 3 feet below Surface. Parts per 100,000.

1905.	July 13.	July 20.	Aug. 31.	Sept. 7.	Sept. 15.	Sept. 21.	Oct. 9.	Oct. 13.	Oct. 19.	Oct. 26.	Nov. 2.	Nov. 10.	Nov. 17.	Dec. 7.	Dec. 14.	Dec. 21.
Total solids	—	122·2	95·0	90·1	94·7	—	99·1	94·0	105·7	100·6	96·9	92·1	116·8	111·0	104·1	103·9
Solids in suspension	—	—	—	Nil	1·6	—	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0·1	Nil
,, organic	—	—	—	,,	0·6	—	,,	,,	,,	,,	,,	,,	,,	,,	0·1	,,
,, mineral	—	—	—	,,	1·0	—	,,	,,	,,	,,	,,	,,	,,	,,	Nil	,,
Chlorine	9·8	11·0	8·6	9·0	9·0	9·6	10·2	10·0	9·3	10·0	9·0	9·2	9·0	9·2	9·0	9·8
Free ammonia	0·013	0·005	0·006	0·007	0·004	0·029	0·012	0·004	0·005	0·004	0·008	0·004	0·025	0·006	0·009	0·003
Albuminoid ammonia	0·045	0·028	0·021	0·023	0·024	0·026	0·034	0·025	0·016	0·021	0·021	0·038	0·027	0·062	0·043	0·042
Oxygen absorbed in 4 hours at 80° F.	0·336	0·264	0·216	0·216	0·268	0·256	0·256	0·256	0·200	0·216	0·188	0·216	0·188	0·256	0·296	0·276
Oxygen absorbed in 3 mins. before incubation at 80° F.	—	0·064	0·068	0·028	0·056	0·068	0·028	0·056	0·064	0·068	0·056	0·064	0·052	0·056	0·096	0·076
Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	—	—	—	—	—	0·040	0·056	0·040	0·052	0·056	0·040	0·052	0·028	0·080	0·080	0·052
Nitric nitrogen on day of collection	—	2·00	1·82	2·17	2·17	1·67	1·67	2·17	1·67	1·67	1·54	2·00	1·43	2·00	1·27	1·00
,, ,, day after collection	1·05	2·50	2·00	1·88	1·85	1·61	2·63	2·00	2·17	2·23	1·82	1·54	2·63	1·32	1·47	1·00
Nitrous nitrogen on day of collection	0·066	0·022	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
,, ,, day after collection	0·12	0·018	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
Column necessary to obscure test lines (inches)	7·5	6·5	Over 2′	Over 2′	20·0	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′

Filter Effluent, 4·5 feet below Surface. Parts per 100,000.

1905.	July 13.	July 20.	Aug. 31.	Sept. 7.	Sept. 15.	Sept. 21.	Oct. 9.	Oct. 13.	Oct. 19.	Oct. 26.	Nov. 2.	Nov. 10.	Nov. 17.	Dec. 7.	Dec. 14.	Dec. 21.
Total solids	—	124·7	95·0	93·8	94·2	—	95·0	96·1	110·8	104·8	91·3	98·0	122·7	119·6	105·1	112·8
Solids in suspension	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, organic	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, mineral	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chlorine	9·8	10·8	8·8	9·4	9·2	9·6	10·2	10·0	9·6	10·2	9·0	9·0	8·8	9·2	9·2	9·8
Free ammonia	0·075	0·097	0·027	0·031	0·018	0·029	0·018	0·023	0·009	0·096	0·031	0·026	0·092	0·035	0·040	0·043
Albuminoid ammonia	0·032	0·039	0·019	0·022	0·023	0·026	0·026	0·029	0·021	0·025	0·029	0·027	0·024	0·040	0·027	0·029
Oxygen absorbed in 4 hours at 80° F.	0·336	0·300	0·188	0·228	0·256	0·256	0·240	0·240	0·200	0·256	0·228	0·316	0·344	0·268	0·228	0·264
Oxygen absorbed in 3 mins. before incubation at 80° F.	—	0·128	0·068	0·040	0·068	0·068	0·028	0·068	0·064	0·080	0·056	0·076	0·130	0·040	0·040	0·100
Oxygen absorbed in 3 mins. after incubation (3 days) at 80° F.	—	—	—	—	—	0·040	0·040	0·028	0·052	0·068	0·056	0·064	0·00	0·056	0·028	0·00
Nitric nitrogen on day of collection	—	1·56	1·72	1·55	1·82	1·67	1·54	2·17	1·33	1·33	1·25	1·67	1·92	2·86	1·51	1·67
,, ,, day after collection	1·82	2·08	1·82	1·15	1·54	1·61	2·23	2·22	2·50	1·67	1·72	1·88	4·36	2·50	1·35	1·43
Nitrous nitrogen on day of collection	Nil	0·011	Nil	0·003	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0·011	0·011	0·018	0·022
,, ,, day after collection	Trace	0·007	,,	Nil	,,	,,	,,	,,	,,	0·00	,,	,,	Trace	0·011	0·003	0·014
Column necessary to obscure test lines (inches)	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	Over 2′	8·0	16·0	20·0	22·0

The Relation of the Kidneys to Metabolism.—Preliminary Communication.

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(From the Gordon Laboratory and Physiological Laboratory, Guy's Hospital.)

The effects following on the removal of large portions of the kidneys of dogs were studied by Rose Bradford in 1892. In the first place, he found that animals deprived of three-quarters or more of their total kidney weight rapidly wasted, and died in two or three weeks, or even less; and, although they often refused food, they passed daily an amount of urea almost or quite equal to that passed by the same animals on full diet before operation. Secondly, he observed that after the removal of a portion of one kidney, the urine became more abundant and more dilute, and the dogs were apparently unable to excrete a concentrated urine; this effect was intensified by the subsequent removal of the opposite kidney. Bradford concluded that the kidneys in some manner normally control the nitrogenous metabolism, and that in the absence of sufficient kidney substance this metabolism becomes excessive.

In view of their extreme importance, a repetition of these experiments appeared advisable; it was hoped, too, that more complete analysis of the urine might throw some light on the course of nitrogenous metabolism as a whole. This paper contains a preliminary account of our observations.

Methods.

Cats were used for these experiments. They were anæsthetised with chloroform and ether, the anæsthesia being maintained by ether throughout the operation.

The abdomen was opened in the middle line, and the kidney drawn up into the wound; the capsule was opened, and stripped off the kidney. The renal vessels were digitally compressed, a wedge cut out of the kidney, and the cut surfaces brought into apposition and kept together by two or three sutures passing deeply through the kidney substance. The kidney was then replaced in its capsule and the latter was closed by a continuous suture; the kidney was returned to its place and the abdominal wound closed. No vessels were ligatured, since forceps are so apt to damage the kidney substance: a little blood collects between the kidney and capsule, where it clots and checks further loss of blood.

Two or three weeks later the opposite kidney was removed under ether anæsthesia through an abdominal incision.

The animals were kept in cages whose floor consisted of a wire netting; the cages stood upon a zinc plate, sloping towards the centre, which was perforated by a small hole. The urine ran down the zinc sheet through the hole into a basin containing a little chloroform. The urine and fæces were collected every 24 hours, and very great care was taken to keep the urine free from food or fæces.

The cats were kept at a fairly even, moderate temperature, and were allowed some exercise daily in the laboratory.

The food was measured daily, and any food left over was also measured. Their diet consisted of minced meat, milk and water; the amount of the latter, of course, was not restricted.

The total nitrogen in the meat and milk was estimated by Kjeldahl's method. A considerable amount of meat was minced, and mixed into a uniform mass; a sample of this was analysed, and the rest kept in an ice chest; the cats received a definite quantity each day.

The urine was analysed daily; the fæces were collected for several days, and the total nitrogen present estimated. The total nitrogen of the urine and fæces was estimated by Kjeldahl's method, the urea by a modification of Folin's method, the ammonia by Schaffer's method, and the creatinin by the colorimetric method of Folin. All the analyses were made in duplicate.

Results.

The effects of removing portions of the kidneys may be grouped as follows:—Firstly, the general conditions of the animals including changes in body weight; secondly, the nitrogenous metabolism; thirdly, alterations in the amount and concentration of the urine.

I. *The Condition of the Animals.*—After the first operation, the cats usually drank a little milk on the same day, but for several days their appetite was poor, and they lost weight. Eventually they ate very well, and appeared to be more hungry than normal cats. For a day or two the urine contained blood, and for another two or three days albumen was present.

Three cats survived the second operation for some time; they rapidly recovered from the anæsthesia, and showed very little shock.

Two of the three cats ate very little after the second operation, and refused food altogether during the last few days of life; the third cat ate well for 10 days, when its appetite also failed.

Vomiting and diarrhœa were observed in all the cats, and they seemed very susceptible to cold. They acquired a very foul breath, and soreness of the lips and gums; their fur came off very rapidly.

Cat I lived six days, Cat II lived seven days, and Cat III lived 17 days after the second operation. The rectal temperature was maintained at 100° or more until the last few days of life.

The Body Weight.—It will be seen from the following table that normal cats lose weight when kept in confinement, notwithstanding some exercise daily. The loss of weight, however, is less than that observed in Rose Bradford's dogs, most of which lost 10 per cent. or more of their initial weight before the first operation. In our experiments, the cats were not put into the collecting cage before the first operation, so that this initial loss of weight was avoided.

Table I.

	Initial weight.	Final weight.	Loss.	Time in cage.
	grammes.	grammes.	Per cent.	days.
Normal cat.........	3650	3580	1·9	19
Normal cat.........	2300	2140	6·9	8

The effect of the two operations upon the weight of the cats is shown in the following table:—

Table II.

	Initial weight, first operation.	Weight at time of second operation.	Loss.	Final weight.	Loss.	Life after second operation.
	grammes.	grammes.	Per cent.	grammes.	Per cent.	days.
Cat I	2420	2120	12·3	1600	33·8	6
Cat II	2880	2550	11·4	1840	36·1	7
Cat III	2500	2220	11·2	1640	34·4	17

The final percentage loss of weight is much the same, and was reached in Cat III in 17 days, and in a much shorter time in the other cats, because Cat I ate well for 10 days, whereas the others ate very little after the second operation. These figures correspond closely with those of Rose Bradford; he also found that the animals lived much longer after the second operation when they took food.

II. *The Nitrogenous Metabolism.*—The total nitrogenous metabolism was determined in three cats before and after the second operation; the urinary nitrogen was also estimated in one of these cats before the first operation. The results are summarised in the following protocols:—

Cat I.—October 12. Weight 2420 grammes. Removed part of left kidney, weight, 6·3 grammes.

Date.	Weight.	Total N in urine.	N as urea.	N as ammonia.	N as creatinin.	N in food.
	grammes.	grammes.	Per cent.	Per cent.	Per cent.	grammes.
October 26......	2130	1·69	—	3·9	1·6	3·0
„ 27......	2160	3·44	90	4·3	1·7	3·0
„ 28......	2090	1·09	—	2·2	1·5	2·6
„ 29......	Removed right kidney: weight 10·5 grammes.					
„ 30......	2050	0·94	74	2·6	2·3	0·83
„ 31......	1950	0·86	90	1·6	2·4	
November 1 ...	1820	0·84	—	3·6	2·7	
„ 2 ...	1770	1·73	—	2·0	2·0	
„ 3 ...	1660	1·02	70	6·0	1·6	
„ 4 ...	1600	Found dead.				

	N intake.		N output.		Fæces.
	Total.	Average.	Total.	Average.	
	grammes.	grammes.	grammes.	grammes.	gramme.
After first operation— 3 days' period............	8·5	2·84	6·22	2·07	0·59
After second operation— 6 days	1·2	0·2	5·71	0·95	0·52

Post mortem.—No evidence of sepsis. Some subcutaneous fat still present. Remainder of kidney weighed 3·8 grammes.

Total kidney weight .... = 20·6 grammes.

Amount after second operation (3·8 grammes) = less than $\frac{1}{5}$ total kidney weight.

= 1·5 grammes per kilo.

Cat II.—October 19. Weight, 2880 grammes. Removed part of left kidney, 8·8 grammes.

Date.	Weight.	Total N in urine.	N as urea.	N as ammonia.	N as creatinin.
	grammes.	grammes.	Per cent.	Per cent.	Per cent.
October 26	2600	2·65	73·9	3·8	1·4
November 1	2510	3·08	92·8	3·3	1·7
„ 3	2550	3·43	79·9	—	1·2
„ 4	2550	3·59	—	3·8	1·4
„ 5	Removed right kidney, weighing 14·1 grammes.				
„ 6	2450	1·10	88·2	3·4	2·2
„ 7	2380	1·69	84·0	1·6	2·1
„ 11	2000	1·79	89·9	2·0	1·2
„ 12	Cat moribund. T. 95° ·0. Killed by chloroform. Weight 1840 grs.				

	N intake.		N in urine.		Total fæces.
	Total.	Daily average.	Total.	Daily average.	
	grammes.	grammes.	grammes.	grammes.	grammes N.
After first operation— Period of 10 days	39·7	3·9	29·01	2·9	2·26
After second operation— 6 days	4·46	0·74	8·78	1·46	1·0 (approx.)

Post mortem.—Remainder of kidney adherent to spleen, normal in appearance, very little omental fat; none visible elsewhere. Muscles not obviously wasted. No evidence of sepsis.

Total kidney weight...... = 29·6 grammes.
Weight found *post mortem* = 6·7 „
= $\frac{2}{9}$ total kidney weight.
= 2·3 grammes per kilo.

Cat III.—September 4. Weight, 2500 grammes. Removed part of left kidney—4 grammes.

Date.	Weight.	Total N in urine.	N as urea.	N as NH_3.	N as creatinin.	N intake.
	grammes.	grammes.	per cent.	per cent.	per cent.	grammes.
September 11......	2140	3·52	—	3·1	1·3	10·4
„ 12......	2120	4·51	88·8	4·5	—	7·6
„ 16......	2220	5·12	90·1	3·6	1·3	7·3
„ 19......	Removed right kidney, wt. 10·25 grammes; wt. of cat, 2220 grammes.					
„ 23......	2020	3·26	75·0	9·9	2·0	9·0
October 2	1880	1·13	83·1	10·0	1·7	nil
„ 5	1670	0·89	—	2·0	1·1	nil

After the second operation, the cat ate well for 12 days; after that it ate very little and wasted rapidly. It was moribund on October 6, and was killed by chloroform. Weight at death 1640 grammes.

Post mortem.—There was no sepsis; very little fat was visible in the subcutaneous tissues.

Total kidney weight...... = 20·65 grammes.
Amount left *post mortem* = 6·4 „
= less than $\frac{1}{3}$ total kidney weight.
= 2·5 grammes per kilo.

	Total N intake.	Daily average.	Total N in urine.	Daily average.
	grammes.	grammes.	grammes.	grammes.
After first operation—				
3 days' period	25·2	8·4	11·3	3·77
After second operation—				
First 6 days	23·4	3·9	12·86	2·14
Second 6 days	24·2	4·03	16·0	2·6
Last 4 days	0	0	3·72	0·93

The nitrogen intake for Cat III is not absolutely exact, since the meat and milk were not analysed every day.

The clinical condition of these cats was apparently identical with that of Rose Bradford's dogs; in both cases, the second operation was usually followed by rapid wasting. In two of the three cats considerably more than three-quarters of the total kidney weight was removed; in the third cat rather less than three-quarters was removed, but since the animal died, one may conclude that cats need rather more kidney than dogs in order to maintain life. In this connection it may be noted that in cats the average kidney weight is 9·1 grammes per kilo., as compared with 6·7 grammes per kilo. in dogs.

There is no doubt, then, that the experimental conditions of Bradford's dogs were strictly reproduced. Yet one of the cats showed no increased output of nitrogen after the second operation; in one cat the increase of nitrogen first occurred five days after the operation, when the cat had lost 28 per cent. of its weight, and was almost moribund; and in the other cat the increase occurred four days after the operation, when the loss of weight was 22·5 per cent.

In none of the cats did the nitrogen output after the second operation ever reach that observed after the first operation, and it never greatly exceeded that found in normal fasting cats. The greatest daily output of urinary nitrogen after the second operation, when the cats refused food, was 1·79 in Cat II on the last day of life. For normal cats kept without food for 24 hours the output of urinary nitrogen is about 1 gramme and the loss of weight about 100 grammes.

The protocols, moreover, show that the second operation has no appreciable effect upon the percentage of urea, ammonia and creatinin relatively to the total nitrogen. There is no increase in the output of creatinin (on a milk diet) such as might be expected if muscular metabolism were excessive.

We find, therefore, that the removal of three-quarters of the kidney weight of cats has no influence upon their nitrogenous metabolism until the last two or three days of life. This terminal increase of nitrogen only occurs when the cats have lost 22 per cent. or more of their body weight, and are obviously in a state of inanition. The two cats which showed this terminal increase of urinary nitrogen ate very little after the second operation, and wasted rapidly.

The effect of starvation upon the body weight and urinary nitrogen of normal animals has been investigated by Voit, Schöndorff, Kaufmann, and others. It is clear, from a comparison of their results with the condition of our cats, that the condition of starving animals is identical with that of cats which refuse food after the second operation on the kidneys.

There is in both cases a progressive loss of weight, a fall of temperature during the last few days of life, and usually a terminal rise in the output of nitrogen in the urine. E. Voit and others have shown that, in starvation, the nitrogen output may increase several days before death, and that the time of its onset is directly related to the amount of fat originally possessed by the animal. A very fat animal may never exhibit the increase of nitrogen, whereas, if the original store of fat is small, a very few days of starvation suffice to evoke an increased output of nitrogen. For example, a starving dog, observed by Schöndorff, showed an increased output of nitrogen when it had lost 20 per cent. of its weight; in our cats the nitrogen rose coincidently with a loss of 22 per cent. or more of the body weight.

We consider, therefore, that the wasting, fall of temperature and final increase of urinary nitrogen occur simply because the cats refuse food after the second operation; they die from inanition, and the rise of urinary nitrogen, when it occurs, merely means that the animal's store of fat is much diminished, and that more energy must be supplied by proteid breakdown.

We consider, further, that the same explanation fully accounts for the results obtained by Rose Bradford, and that there is no evidence from his experiments that the kidneys directly influence nitrogenous metabolism.

It may be added that Dr. Pembrey has investigated the gaseous exchange of these cats; he finds that his results are such as would be given by animals in a state of inanition.

III. *The Amount and Concentration of the Urine.*—The amount and specific gravity of the urine of cats varies widely according to their diet. On a meat diet the urine is concentrated; on a milk diet it is abundant and dilute. Making allowance for variations of diet, we find no evidence that the amount and specific gravity of the urine are necessarily modified by either the first or second operation. Only one cat almost invariably passed abundant quantities of dilute urine after the first operation; the others often passed a concentrated urine. In this respect our results are at variance with those of Rose Bradford; and it may be pointed out that the effect, even in dogs, is not a constant one, since in half of Bradford's experiments the urine was no more abundant after the second than after the first operation.

It may be concluded, therefore, that the increase in quantity and decrease in concentration of dog's urine, after operations on the kidneys, is an incidental rather than an essential result.

Table III.

	Nature of food.	Urine.		
		Amount.	Sp. gr.	Total N.
		c.c.		grammes.
Normal cat	Meat	112	1046	5·4
	Bread and milk	310	1007	1·6
After first operation—				
Cat I	Meat	128	1040	5·1
	Bread and milk	435	1005	1·55
Cat II	Meat	164	1038	3·44
	Milk	124	1020	2·1
Cat III	Meat and milk	68	1042	2·65
Cat IV	Meat	110	1040	?
Cat V	Meat	43	1054	
	Milk	150	1015	
After second operation—				
Cat I	Milk	90	1020	1·49
	Meat	182	1018	3·02
Cat II	Milk	78	1030	0·94
		81	1018	1·07
Cat III	Meat and milk	100	1019	1·10
		208	1018	2·44

Conclusions.

1. We confirm Bradford's observations that removal of three-quarters or more of the total kidney weight in cats is followed by loss of appetite, wasting, and death within a few days or weeks.

2. We find that in such cats an increased output of nitrogen is not of constant occurrence; and that it takes place only in cats which have lost 22 per cent. or more of their initial body weight at the time of its onset.

3. We conclude, therefore, that the kidneys have no direct influence upon nitrogenous metabolism, and that the increased output of nitrogen is simply the result of inanition, and is of the same nature as that observed in starving animals.

4. We find that, after removal of a portion of one kidney, and also after subsequent removal of the opposite kidney, cats are still able to pass a concentrated urine, and that the amount of the urine is not necessarily increased beyond the normal.

The expenses of this research were partly defrayed by a grant from the Grants Committee of the Royal Society, to whom we desire to express our indebtedness.

REFERENCES.

Bradford, 'Journ. Physiol.,' vol. 23, p. 415, 1898.
Folin, 'Journ. Phys. Amer.,' vol. 13, 1905.
Kaufmann, 'Zeitschrift f. Biol.,' vol. 41, 1901.
Schöndorff, 'Pflüger's Archiv,' vol. 82, p. 60, 1900.
Voit, E., 'Zeitschrift f. Biol.,' vol. 41, p. 550, 1901.

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REFERENCES.

Bradford, 'Journ. Physiol.,' vol. 23, p. 415, 1898.
Folin, 'Journ. Phys. Amer.,' vol. 13, 1905.
Kaufmann, 'Zeitschrift f. Biol.,' vol. 41, 1901.
Schöndorff, 'Pflüger's Archiv,' vol. 82, p. 60, 1900.
Voit, E., 'Zeitschrift f. Biol.,' vol. 41, p. 550, 1901.

Address of the President, Lord Rayleigh, O.M., D.C.L., at the Anniversary Meeting on November 30, 1906.

Since the last Anniversary the Society has sustained the loss of twelve Fellows and two Foreign Members.

The deceased Fellows are :—

Professor Lionel Smith Beale, died March 28, 1906.
Sir Walter Lawry Buller, K.C.M.G., died July 19, 1906.
Charles Baron Clarke, died August 25, 1906.
Right Hon. Sir M. E. Grant Duff, G.C.S.I., died January 11, 1906.
Professor Charles Jasper Joly, died January 4, 1906.
Colonel Sir Alexander Moncrieff, K.C.B., died August 3, 1906.
George James Snelus, died June 18, 1906.
Professor Hermann Johann Philipp Sprengel, died January 14, 1906.
General Sir Henry E. L. Thuillier, C.S.I.
Rev. Canon H. B. Tristram, died March 8, 1906.
Professor Harry Marshall Ward, died August 26, 1906.
Professor Walter Frank Raphael Weldon, died April 13, 1906.

The Foreign Members are :—

Professor Ludwig Boltzmann.
Professor Samuel Pierpont Langley.

On this list are to be found the names of veterans distinguished in many branches of science and in public affairs. One name is a household word in every physical and chemical laboratory. It would be difficult, indeed, to enumerate the investigations which have owed their success to the invention of the Sprengel mercury pump. In other cases, scientific careers still in full activity have, unhappily, been cut short. I allude especially to Joly, Marshall Ward, and Weldon. Even within my term of office our discussions have been enlivened by Weldon's scientific enthusiasm and vigorous polemics.

On the Foreign list are two distinguished names. Professor Ludwig Boltzmann, of Vienna, was perhaps the first Continental physicist to take up the ideas of Maxwell's electric theory of light, of which he had early grasped the scope, and became for many years one of its most emphatic supporters. One of his earliest series of experiments was a determination of the influence of the crystalline quality on the dielectric constant of sulphur, with a view to comparison with its optical double refraction. In the theory of gases he is to be classed along with Clausius and Maxwell as one of the creators of the dynamical theory, on which he became the highest authority. By developing an idea originated by Bartoli he placed Stefan's law of intensity of natural radiation on a theoretical basis, and thus became the pioneer in the modern thermodynamics of radiant energy. He contributed to the advance of physical science by many other investigations, and by his books on Gas Theory, on Electrodynamics, and on Mechanics. I may perhaps be allowed to add that at the time of his unhappy death, Boltzmann's name was before the Council as proposed for one of our medals.

Professor Langley's work was more on the experimental side of physics. In his bolometer he applied electric resistance thermometry to radiation, and was thereby enabled to penetrate further into the important and mysterious region of the ultra-red than had before that time been possible. For this work he received the Rumford Medal in 1886. During the later years of his life his attention was largely occupied with the mechanical problem of flight, and his models attained a considerable measure of success. As Secretary to the Smithsonian Institution at Washington he did much to forward, by his co-operation and advice, all kinds of scientific investigation.

In the Report of the Council there has been laid before you an account of the work of the Council and of various Committees in a very wide field. The investigation of the terrible disease known as Sleeping Sickness has unhappily been marked by the tragic death of Lieutenant Tulloch, who has fallen a victim to his zeal in studying the disease in Uganda. Vigorous efforts are being undertaken to discover some therapeutic remedy for the malady. In the case of Malta Fever, too, the investigation of which was entrusted to the Royal Society by the Colonial Office, good progress has been made. It has been ascertained by the Society's Commission in Malta that the main source of propagation is the milk of infected goats. When this discovery was made the authorities of the island were at once warned of the danger in the milk supply, and the necessary precautions were taken. Since then the number of cases of fever in the hospitals has so greatly diminished as to afford good hope that this disease, which has been so great a scourge in Malta, may ere long be reduced to insignificant proportions or altogether exterminated.

I observe that a movement has been been started in this country in aid of the Greek Anti-Malaria League. Professor Ronald Ross, than whom there is no higher authority, bears witness to the unexpected prevalence of the infection in most of the localities examined, and he is confident that practical results of the highest value would follow expenditure in combating the disease on lines already laid down. Although I speak only from general knowledge, I cannot let this opportunity pass without emphasising my sense of the enormous importance of this class of work. If men knew where their real interests lie, our efforts in this direction would be doubled or quadrupled. In this way discoveries, which the future will certainly bring, might be accelerated by decades, giving health and life to thousands or millions who now succumb. Willing and competent workers would soon offer themselves; the principal obstacle is the want of means.

The preparation of the 'Royal Society Catalogue of Scientific Papers' for the remaining portion of the 19th century, which has proved a task so much more gigantic than can have been contemplated by the originators of the Catalogue nearly half a century ago, has been actively pushed forward. In consequence of the increased expenditure, now at the rate of nearly £2000 a year, the funds available are again approaching exhaustion. The difficulties of the President and Council and of the Catalogue Committee on this subject have once more been promptly resolved by the action of our Fellow, Dr. Ludwig Mond, who, after consultation with the Officers, has again made himself responsible for a further subsidy amounting to £2000 a year for three years. It is hoped that with the balance in hand and other sources of income, including the Handley Fund of the Royal Society, the income of which is devoted to this purpose, this subvention will suffice for the preparation of the work and for passing it through the press. Since the Royal Society took this great national task in hand there has already been spent on it over £23,500, while on each occasion of financial stress Dr. Mond has come forward with the means of relief, his direct contributions, including that just promised, now amounting in the aggregate to £14,000. This great work when published will thus be a tangible memorial of Dr. Mond's practical insistence on the importance of adequate indexes of the vastly increasing literature of science.

Of the activities working under the Royal Society the one with which I have been especially connected is the National Physical Laboratory.

In their Report for the past year the Executive Committee call attention to the loss they have sustained by the deaths of Sir Edward Carbutt and Sir Bernhard Samuelson, both members of the Committee and warm supporters of its work.

The Report shows continued progress. As a result of a memorial to the Chancellor of the Exchequer, signed by about 150 Members of Parliament, the Grant for building and equipment for the year was increased from £5000 to £10,000, and this has enabled the Committee to take in hand some urgently needed extension.

Buildings are now in course of erection for Metrology and for Metallurgical Chemistry, while the Engineering Laboratory is being doubled in area. The two last additions were called for in great measure in consequence of an arrangement with the India Office whereby the testing work required for the Indian Government, hitherto carried on at Coopers Hill, is to be transferred to the Laboratory. The Indian Government provide the testing machine and other appliances required for the work, and, in addition, have intimated their intention of placing in the charge of the Committee the very admirable electrical equipment now at Coopers Hill.

Towards the equipment of the Metallurgical Laboratory the Goldsmiths' Company have made a very generous donation of £1000, while the Governments of New Zealand and Western Australia have contributed £100 each to the equipment of the Metrological Laboratory.

The buildings of the Electrical Laboratory have been completed, and were formally opened by the Right Hon. R. B. Haldane in June last. On this occasion many representatives of electrical industry from various parts of the world, who were attending the special conferences of the Institution of Electrical Engineers, were present, and joined in the inspection of the Laboratory.

After the meeting Sir John Brunner announced his intention of subscribing £5000 towards the equipment of the various buildings now in hand.

A number of important researches, a list of which is given in the Report, have been published during the year, and others are in a forward state of preparation for publication. Dr. Carpenter and Mr. Edwards have completed the first part of their research into the properties of the copper-aluminium alloys for the Alloys Research Committee. Dr. Stanton and Mr. Bairstow have read a paper before the Institution of Civil Engineers on the effect of alternating stresses on steels. The work on electrical standards with the Ampère Balance, which has a special interest for myself, is well advanced. In the hands of Mr. Smith the measurement of the electrolytic deposits of silver, which represent the whole passage of electricity through the apparatus, has attained a remarkable precision and, what is perhaps more important, some anomalies met with at first seem to be on the road to elucidation. Mr. Campbell has made progress with his research on hysteresis in steel sheets, and Dr. Harker is engaged with interesting investigations in high-temperature thermometry.

Changes in the staff have taken place. Dr. Caspari resigned his position in order to take up private practice, while Dr. Carpenter has been appointed the first Professor of Metallurgy in the Victoria University of Manchester. The arrangements with the Indian Government have necessitated an alteration in the metallurgical division, and the Committee have thought it right to constitute a Department of Metallurgy and Metallurgical Chemistry, with a Superintendent in charge of the work. They have been fortunate in securing the services of Mr. W. Rosenhain as the first occupant of the post.

A question of importance has arisen as to the performance at the Laboratory of tests, partaking of a routine character, on the physical and mechanical properties of specimens of material. To this objection has been taken on the ground of competition with the work of private establishments. In one of its aspects the question is financial. But the Executive Committee are of opinion that, even if the pecuniary loss were recouped, the efficiency of the Laboratory would suffer from the abandonment of this work. While anything like unfair competition with private establishments should be avoided, the execution of tests is good practice for the staff, and tends to keep them in touch with the manufacturers and with the practical problems which may demand examination. In view of the difference of opinion that has manifested itself, the Treasury has decided to appoint a small committee to inquire into the working of the Laboratory, with a special reference to this question.

On a former occasion, my distinguished predecessor, Sir William Huggins, called attention to some of the more important matters on which the Society in the past had initiated, supported, or given advice about scientific questions in connection with the State, and in other ways had made its influence felt strongly for the good of the country. It would hardly become me, with my short experience of the working of the Society, at least in recent years, to pursue this theme. The function of the Society which lies most open to the observation of an incoming President is that exercised at the ordinary meetings. I am impressed with the difficulty, arising out of the ever-increasing specialisation of science, in rendering really useful the reading of papers and discussions thereupon. It is, of course, felt more severely in a Society like our own, which embraces within its scope the whole scientific field. It not infrequently happens that a paper is addressed to an audience among whom there is no one competent to follow the detailed observations and reasonings of the author. I am sometimes reminded of a saying of Dalton's on similar occasions at Manchester, quoted by Sir Henry Roscoe in his genial and entertaining 'Reminiscences': "Well, this is a very interesting paper for those that take any interest in it." A little more discretion on the part of readers of papers in having regard to the composition of their actual audience would be helpful here. In some cases experimental illustration would bring home to a larger number what is followed with difficulty from a merely verbal statement. But I am afraid that no complete remedy is within reach.

Increase of specialisation, however inconvenient in some of its aspects, is, I suppose, a necessary condition of progress. Sometimes a big discovery, or the opening up of a new point of view, may supersede detail and bring unity where before there was diversity, but this does not suffice to compensate the general tendency. Even in mathematics, where an outsider would probably expect a considerable degree of homogeneity, the movement towards diversity is very manifest. Those who, like myself, are interested principally in certain departments, and can look back over some 40 years, view the present situation with feelings not unmixed. It is disagreeable to be left too far behind. Much of the activity now displayed has, indeed, taken a channel somewhat remote from the special interests of a physicist, being rather philosophical in its character than scientific in the ordinary sense. Much effort is directed towards strengthening the foundations upon which mathematical reasoning rests. No one can deny that this is a laudable endeavour; but it tends to lead us into fields which have little more relation to natural science than has general metaphysics. One may suspect that when all is done fundamental difficulties will still remain to trouble the souls of our successors. Closely connected is the demand for greater rigour of demonstration. Here I touch upon a rather delicate question, as to which pure mathematicians and physicists are likely to differ. However desirable it may be in itself, the pursuit of rigour appears sometimes to the physicist to lead us away from the high road of progress. He is apt to be impatient of criticism, whose object seems to be rather to pick holes than to illuminate. Is there really any standard of rigour independent of the innate faculties and habitudes of the particular mind? May not an argument be rigorous enough to convince legitimately one thoroughly imbued with certain images clearly formed, and yet appear hazardous or even irrelevant to another exercised in a different order of ideas? Merely as an example, there are theorems known as "existence-theorems" having physical interpretations, the object of which is to prove formally what to many minds can be no clearer afterwards than it was before. The pure mathematician will reply that even if this be so, the introduction of electrical or thermal ideas into an analytical question is illogical, and from his own point of view he is, of course, quite right. What is rather surprising is that the analytical argument should so often take forms which seem to have little relation to the intuition of the physicist. Possibly a better approach to a reconciliation may come in the future. In the meantime we must be content to allow the two methods to stand side by side, and it will be well if each party can admit that there is something of value to be learned from the point of view of the other.

In other branches, at any rate, the physicist has drawn immense advantage from the labours of the pure analyst. I may refer especially to the general theory of the complex variable and to the special methods which have been invented for applying it to particular problems. The rigorous solution by Sommerfeld of a famous problem in diffraction, approximately treated by Fresnel, is a case in point. We have moved a long way from the time when it was possible for the highest authority in theoretical optics to protest that he saw no validity in Fresnel's interpretation of the imaginary which presents itself in the expression for the amplitude of reflected light when the angle of incidence exceeds the critical value. In this connection it is interesting to remember that, in his correspondence with Young, Laplace expressed the opinion that the theoretical treatment of reflexion was beyond the powers of analysis. The obvious moral is that we are not to despair of the eventual solution of difficulties that may be too much for ourselves.

As more impartially situated than some, I may, perhaps, venture to say that in my opinion many who work entirely upon the experimental side of science underrate their obligations to the theorist and the mathematician. Without the critical and co-ordinating labours of the latter we should probably be floundering in a bog of imperfectly formulated and often contradictory opinions. Even as it is, some branches can hardly escape reproaches of the kind suggested. I shall not be supposed, I hope, to undervalue the labours of the experimenter. The courage and perseverance demanded by much work of this nature is beyond all praise. And success often depends upon what seems like a natural instinct for the truth—one of the rarest of gifts.

MEDALLISTS, 1906.

The Copley Medal is awarded to Professor Elias Metchnikoff, For. Mem. R.S., on the ground of his distinguished services to zoology and to pathology, particularly for his observations on the development of Invertebrates and on phagocytosis and immunity. From 1866 to 1882 Professor Metchnikoff's work was exclusively zoological, and mainly during that period he produced a series of brilliant memoirs dealing with the early development and metamorphoses of Invertebrates.

Although his name stands in the first rank of investigators of these subjects, the most celebrated of his discoveries are those relating to the important part played by wandering mesoderm cells and white blood-corpuscles in the atrophy of larval organs, and in the defence of the organism against infection by Bacteria and Protozoa. It was on these researches that he based his well-known 'Phagocyte Theory.' Metchnikoff's fundamental observations were made in Messina in 1882, and were published in the following year. In these he showed that the absorption and disappearance of the embryonic organs of Echinoderms were effected by wandering mesoderm cells, which devoured and digested the structures which had served their purpose and become effete. The observation that white blood-cells accumulate in an inflamed area after infection by Bacteria suggested that these cells might also devour and thus destroy the invading microbes, and that the process of inflammation was really a physiological and protective reaction of the organism against infection. The study of the infection of *Daphnia* by *Monospora bicuspidata* entirely justified this prediction. The account of the phenomena of infection as seen in this transparent Crustacean was published in 'Virchow's Archiv' (vol. 96) in 1884, while, later in the same year, Metchnikoff published another paper extending these observations to Vertebrates, and showing the universal applicability of his generalisation as to the essential character of the inflammatory process.

During the twenty years which have elapsed since the publication of the 'Phagocyte Theory,' Metchnikoff, with the assistance of a host of pupils and disciples from all parts of the world, has been continuously engaged in the study of the reaction of the organism against infection, and in investigating the essential features of immunity in the light of the illuminating generalisation laid down in 1884.

Though of limited range, and therefore inferior in scientific importance to the more fundamental researches carried out by him previously, Metchnikoff's recent work on infection by the micro-organism of syphilis and the attainment of protection and immunity against this disease may be mentioned on account of its important practical applications.

It is not too much to say that the work of Metchnikoff has furnished the most fertile conception in modern pathology, and has determined the whole direction of this science during the last two decades.

The Rumford Medal is awarded to Professor Hugh Longbourne Callendar, F.R.S., for his experimental work on heat.

Professor Callendar has devoted his attention chiefly to the improvement of accurate measurement in the science of heat by the application of electrical methods. His first paper "On the Practical Measurement of Temperature," 'Phil. Trans.,' 1887, paved the way for the application of the electrical resistance thermometer to scientific investigation. In a later paper, written in conjunction with Griffiths, "On the Boiling Point of Sulphur, etc.," 'Phil. Trans.,' 1891, the application of his method was further extended, and a simple method of standardisation was proposed. In continuation of this work Professor Callendar has written a number of subsidiary papers dealing with details of construction of instruments, and applications to special purposes. The results of this thermometric work have since been confirmed by Chappuis and Harker, 'Phil. Trans.,' 1889, at the Bureau International, Paris, and by other observers, and are now generally accepted.

More recent developments in accurate electrical thermometry have been described by Professor Callendar in later papers. He has also devised a special type of "gas-resistance" thermometer, depending on the increase of viscosity of a gas with temperature, which is the exact analogue of the electrical resistance thermometer, and possesses peculiar advantages for high-temperature measurements.

The application of electrical resistance thermometers and thermo-couples to the observation of rapid variations of temperature has been utilised by Professor Callendar in the study of the adiabatic expansion of gases and vapours, and in the observations of the cyclical changes of temperature of the steam and of the cylinder walls in a steam-engine. The latter research was undertaken in conjunction with Professor Nicholson, with a view to elucidate the theory of cylinder-condensation.

The researches of Rowland and other experimentalists on the specific heat of water, and the mechanical equivalent of heat, had shown that grave uncertainties affected the value of this most fundamental physical constant, which could not be removed satisfactorily without a complete investigation of the variation of the specific heat of water between 0° and 100° C. Professor

Callendar devised a continuous electrical method of attacking this problem, possessing many important advantages as compared with older methods. He was assisted by Dr. Barnes in carrying out this work, the results of which form the subject of papers by Callendar and Barnes in the 'Phil. Trans. Roy. Soc.,' 1901. As an illustration of the probable accuracy of their results it may be observed that, whereas by any of the older formulæ accepted for the variation of the specific heat of water the values of Rowland and of Reynolds and Moorby for the mechanical equivalent are seriously discordant, they are brought into perfect agreement by the work of Callendar and Barnes.

In the subject of conduction of heat Professor Callendar has contributed many original methods described in various minor papers, and, in addition to the thermal investigations with which his name is chiefly associated, has carried out some purely electrical researches.

One of the Royal Medals has been awarded, with the approval of His Majesty, to Professor Alfred George Greenhill, a Fellow of the Society, on account of the number and importance of his mathematical investigations produced between the year 1876 and the present time. They embrace a variety of mechanical and physical subjects, including dynamics, hydromechanics, electricity, and gunnery. He is the author of two treatises on hydromechanics, both remarkable for originality of treatment.

The subject, however, to which he has devoted most time and attention is the theory of elliptic functions. His work on this subject may be placed in two classes: (1) Investigations in which he has extended the subject into new fields, as in the series of memoirs on the "Transformation and Complex Multiplication of Elliptic Functions," contributed to the 'Proceedings of the London Mathematical Society' (vols. 19, 21, 25, 27), and in the memoir on the "Third Elliptic Integral and the Ellipsotomic Problem," in the 'Phil. Trans.' (vol. 203). (2) Applications to Mechanical Problems, mainly dynamical, for purposes of calculation or illustration. In this class may be placed his treatise on the Elliptic Functions, as well as numerous papers in journals and the proceedings of scientific societies.

All Professor Greenhill's work is characterised by much originality, and by a rare power and skill in algebraic analysis.

His Majesty has also approved the award of a Royal Medal to Dr. Dukinfield Henry Scott, also a Fellow of the Society, for his investigations and discoveries in connection with the structure and relationship of fossil plants. Dr. Scott began the very important work which he has accomplished in this subject by helping the late distinguished palæobotanist, Professor W. C. Williamson. In this co-operation he greatly enhanced the value of Williamson's work. He not only added many new discoveries, but, what was more important, demonstrated the value of the work in relation to phylogeny.

Dr. Scott has since added much of first-rate importance. He has discovered and elucidated many important types, his work constituting a most valuable acquisition to botany from the evolutionary point of view. It is not only in the accurate investigation of difficult structures that Dr. Scott has been so successful; not the least of his merits lies in the philosophical treatment of the problems suggested by his discoveries. His position as one of the leading palæobotanists in the world is well recognised. He has, both by his personality and by his writings, exercised a well-marked and widespread influence on the work of other botanists. The fact that he has created in this country a vigorous school of palæobotanists may be regarded as an additional claim for the honour now conferred upon him.

The Davy Medal is given to Professor Rudolf Fittig, Professor of Chemistry in the University of Strassbourg, who began to publish scientific work as early as 1858 and in 1864 discovered the method for the synthesis of hydrocarbons homologous with benzene, which has ever since borne his name. Up to about 1880 he worked chiefly on benzene derivatives, but his attention was gradually attracted to the study of lactones and acids, both saturated and unsaturated, which has largely formed the subject of his numerous published papers down to the present day.

Fittig has been a remarkably active worker. The Royal Society Catalogue contains under his name alone 96 papers, and, jointly with students and others, 71 more down to 1883. Since that time a number about equally large has been recorded in the indexes of the chemical journals. The work of Fittig and his students on lactones and acids, and particularly the intermolecular changes which many unsaturated acids undergo, may be said to be classical, and it has had an important influence on the progress of theoretical chemistry.

The Darwin Medal has been awarded to Professor Hugo de Vries, For. Mem. R.S. Professor de Vries has made a series of important discoveries in connection with the manner in which new races of organisms may originate, and he has materially extended and systematised our knowledge of the laws affecting the results of hybridisation. His work is the outcome of very extensive experiments that have been carried on for many years. He has stimulated numerous investigators, both in Europe and in America, to extend these enquiries; and the results already obtained are of great importance, both from a theoretical and from a practical point of view. De Vries' work has exercised considerable influence on other branches of biology, and has suggested new lines of investigation in many directions.

Mrs. W. E. Ayrton is the recipient of the Hughes Medal, which is awarded for original discovery in Physical Sciences, particularly electricity and magnetism, or their applications. Her work on the Electric Arc has been described in a paper published in the 'Philosophical Transactions,' and in various other publications.

Mrs. Ayrton's investigations cover a wide area. She discovered the laws connecting the potential difference between the carbons of an arc with the current and with the distance between them, and proved these to apply not only to her own experimental results but to all the published results of previous observers. Dealing with the modifications introduced into the arc by the use of cores in the carbons, she found the causes of these modifications. The peculiar distribution of potential through the arc was traced, and its laws were discovered by her.

Having found the conditions necessary for maintaining a steady arc, and for using the power supplied to it most efficiently, she was able to explain the cause of "hissing," and the causes of certain anomalies in the lighting power of the arc.

For the past four years Mrs. Ayrton has been engaged in investigating the causes of the formation of sand ripples on the seashore.

Further Observations on the Effects produced on Rats by the Trypanosomata of Gambia Fever and of Sleeping Sickness.

By H. G. Plimmer, F.L.S.

(Communicated by Dr. C. J. Martin, F.R.S. Received November 21,—Read December 13, 1906.)

[Plate 1.]

In January, 1905, I published* a note of some experiments with the above-named strains of trypanosomata, and I have continued the experiments up to the present time, and have embodied the total results in this paper.

1. *Gambia Fever.*

The number of rats inoculated with this strain of trypanosomata was altogether 129 in 90 successive inoculations. In all of these rats, with the exception of the five mentioned below, trypanosomata were present in the blood from two to three weeks after inoculation (average time 18 days) until death; generally in good numbers, and increasing to large numbers for some days before death. The spleen was, in these cases, very much enlarged, and there was considerable blood destruction, with large numbers of polychromatophile corpuscles, these being the most obvious gross signs.

In the first 14 of these rats, as noted in the paper referred to above, no paralytic symptoms were present, but out of the total number inoculated, five became paraplegic, and the course of the disease in these animals differed from that in the remaining 124.

In the series, the numbers of the paraplegic rats were 35, 42, 68, 101, 102, and there were no perceptible differences in their conditions and those of the other rats used.

The first rat which became paralysed (No. 35, of the 25th inoculation) was inoculated on March 17, 1905, and on the 31st of the same month both hind legs became paralysed. No trypanosomata were found in the blood until April 18, and from that time till death, which took place on April 28, only a few were to be found in ordinary blood films. The second rat (No. 42, of the 28th inoculation) was inoculated on April 13, 1905, from the blood of another rat which had been 20 hours in the stomach of a leech, in which some of the trypanosomata will live for at least 36 hours. The rat became paraplegic on June 15, at which time no trypanosomata could be found in ordinary blood films. The rat was killed on June 16, and a few trypanosomata were found both in the centrifugalised blood and in smears made from the spinal cord. The third rat (No. 68, of the 42nd inoculation) was inoculated on September 26, 1905, and became paraplegic on December 13. No trypanosomata could be found in ordinary blood films in 17 examinations made during the disease, nor at the time of death. The animal died on January 3, 1906, when a few trypanosomata were found in the centrifugalised blood, and in smears from the spinal cord. The fourth and fifth rats (Nos. 101 and 102, of the 70th inoculation) were inoculated on May 26, 1906, and on July 17 No. 101 became paralysed in the right hind leg, and on July 18 No. 102 also became paralysed in the right hind leg. Both of these rats had a good number of trypanosomata in their blood of the ordinary long, very motile kind. On July 19 No. 101 became completely paraplegic, and died on the 22nd. No. 102 died on July 23, without the other leg becoming paralysed.

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* 'Roy. Soc. Proc.,' vol. 74.

The following table gives a comparative outline of these five experiments:—

Rat.	Duration of disease.	Appearance of paralytic symptoms.	Trypanosomata in blood during life.
35	41 days	14th day	Very scanty.
42	64 days, rat killed...	63rd day	Not found; few found *post mortem* in centrifugalised blood.
68	102 days	78th day	Ditto.
101	57 days	52nd day	Good number.
102	58 days	53rd day	Ditto.

It will be noted that, in Rats Nos. 101 and 102, trypanosomata were present in good numbers in the blood, but in those cases (Nos. 42 and 68) in which the paralysis was most marked, and which, to all appearances, were exactly like rats paralysed after inoculation with the Sleeping Sickness strain of trypanosomata, they were absent, or at any rate so few in number that they could only be found in centrifugalised blood after death. From all of these rats inoculations were made, with the constant result that the disease in the inoculated animals reverted to the generalised form mentioned above as characteristic of this strain of Gambia Fever.

This strain of trypanosomata was also inoculated into a monkey, which lived 33 days after inoculation, this being three days longer than the average time of the 14 monkeys I have inoculated with the Sleeping Sickness strain. Trypanosomata appeared in the blood 10 days after inoculation, and were of the type of the Sleeping Sickness strain. Rats inoculated from this monkey did not become paralysed; trypanosomata of the Gambia Fever type were plentiful in their blood, and the disease ran the course of that produced by this strain.

2. *Sleeping Sickness.*

In all, 82 rats have been inoculated with this strain of trypanosomata, in 59 direct series of inoculations, and, of these, 22 have been paralysed. In these cases trypanosomata could be found only at intervals and rarely, and sometimes not at all, in ordinary blood films, and in some cases none could be found in the centrifugalised blood *post mortem*; but in cases where prolonged search was made a few were always found. The paralysis occurred most generally in those rats which were inoculated direct from monkeys, and it occurred less and less frequently as the passages got more remote, firstly from the monkeys and secondly from the original monkey. I have succeeded in producing paralysis once as far as the fourth passage from the monkey; generally after the second, in five instances after the first, passage the trypanosomata have become generalised in the blood, and the symptoms and course of the disease have been those of the Gambia Fever strain.

The following table gives an outline of the instance mentioned above, in which paralysis occurred in four successive passages:—

Rats.	Inoculated from	Duration of disease.		Appearance of paralysis after inoculation.		Trypanosomata in blood.	Spleen.
		mths.	days.	mths.	days.		
7*a*	Monkey which was inoculated from the monkey brought home by Col. Bruce	6	7	5	15	None seen	Not enlarged.
7*b*		3	14	2	2	None seen	Not enlarged.
8	Rat 7*a*	6	0	5	2	None seen	Not enlarged.
10*a*	Rat 8	3	23	3	1	Few	Enlarged.
10*b*		4	6	3	0	Few	Enlarged.
13*a*	Rat 10*a*	3	3	Not paralysed		Few in every field	Large.
13*b*		2	0	1	20	Few	Large.
16	Rat 13*b*	2	15	Not paralysed		Plentiful	Very large.

The rats Nos. 7*a* and 7*b* at the head of the above series were inoculated from a monkey which had been inoculated from the monkey Colonel Bruce, F.R.S., brought home from Uganda, this monkey having been inoculated on the spot with cerebro-spinal fluid from a case of advanced Sleeping Sickness.

In rats inoculated from the 10th monkey done in direct succession from Colonel Bruce's monkey, the paralysis occurred only in those inoculated directly from the monkey, the next passage producing trypanosomata and symptoms of the Gambia Fever type, as set forth in the following table:—

Rat.	Inoculated from	Duration of disease.	Appearance of paralysis after inoculation.	Trypanosomata in blood.	Spleen.
66*a*	Tenth monkey in direct succession from Col. Bruce's monkey	Killed at 3 mths. 25 days	2 mths. 22 days	None seen	Not enlarged.
66*b*		4 mths. 2 days	3 mths. 11 days	None seen	Not enlarged.
66*c*		4 mths. 6 days	3 mths. 22 days	None seen	Not enlarged.
68	Rat 66*a*	2 mths. 26 days	None	Few from 15th day after inoculation, increasing till death	Large.
69	Rat 66*b*	2 mths. 1 day	None	Plentiful	Very large.
70	Rat 66*c*	Killed at 1 mth. 20 days	None	Fair number	Large.
71	Rat 69	2 mths. 18 days	None	Plentiful	Very large.

3. *Paralysis after Inoculation with Trypanosomata.*

With regard to the question of paralysis occurring in the rat after inoculation with the above-mentioned particular strains of trypanosomata, it does not appear to have come within the experience of all those who have worked with these particular organisms. For instance, neither Dr. Thomas, of the Liverpool School of Tropical Medicine, nor Dr. Laveran, of Paris, appear to have encountered it in their work with these trypanosomata.

But Lieutenants Tulloch and Gray, in Uganda, have made a number of experiments to test the statement made in my Preliminary Note, and have recorded the same results in a paper published in No. VIII of the Sleeping Sickness Reports of the Royal Society.

In four rats inoculated with the blood of a monkey which had been previously inoculated with cerebro-spinal fluid from Sleeping Sickness patients, paralysis of the hind limbs occurred 10 or more days before death; and in three out of the four rats no trypanosomata could be found in the peripheral blood at the time of death. They also found that rats did not show any signs of infection after inoculation with the blood of Sleeping Sickness patients, which has also been my experience. I inoculated two rats with blood from a case of Sleeping Sickness, in University College Hospital, a year before death, and two more shortly before death, on both of which occasions trypanosomata were present in the patients' blood; the results in both series were negative.

The site of the inoculation apparently determines the locality of the paralysis. In all my experiments, with the exception of four, inoculation was made at the inner side of the hind leg, and the paralysis occurred in the lower limbs. Only in one rat, which lived nearly 11 months, did any weakness of the forelegs show itself; and this was due to extensive degeneration ascending the spinal cord from the lumbar region. The lesions in the spinal cord were also confined more or less to the lumbar region, depending for their extent upon the duration of the disease. In two cases I inoculated the animals on the inner side of the foreleg, and both these animals became paralysed in the front legs, and the degenerative lesions were most marked in the corresponding parts of the spinal cord.

I inoculated two rats in the peritoneum, but in neither did any paralysis occur.

4. *The morphological characters of these two strains of Trypanosomata.*

It is impossible at present to insist that any differentiation based solely upon microscopical observations should be sufficient in the case of organisms so much alike as the trypanosomata. Our methods and observations are neither uniform nor good enough to enable us to make, at the present time by the microscopical method alone, a sufficient differentiation. Until all observers use the finer zoological methods of fixation, etc.,* instead of the barbarous method of drying blood films at present almost exclusively in use, we cannot look for much certainty in the microscopical differentiation of very similar organisms. This must be supplemented by observing the differences in their pathogenic action.

We must not forget, either, the variability of organisms in the same species of animals in the same country, and their still greater variability in different animals in the same, and in other countries; and we must remember the great differences in the physical conditions of an organism which are due to environment. So I would not insist, with our present knowledge, too strongly upon the differences in form in the trypanosomata mentioned above, under the different conditions which have been described. But if preparations are made under the same conditions, and with the same care, it can be seen that the trypanosomata from the cases in which paralysis has occurred (in which very few are found in the blood, the greater number being in the central nervous system) are short and thick, with the flagellum extending very little beyond the body of the organism; they contain a varying number of vacuoles, and sometimes many granules; they move slowly, even when the smear of cord or brain is diluted with normal citrate solution (fig. 3). On the other hand, those found in the generalised cases (the Gambia Fever type) are long, with a long flagellum, and are not vacuolated, and move quickly (fig. 4). I have not found the other form in any of these cases, even when the animal has been long dying. This short, badly staining, vacuolated form is probably due to some condition of environment about which we know nothing, which produces not only changes in the morphology of the organism, but also in its pathogenic effects, for, from the above experiments, it can be seen that the inoculation of these short forms may, in the first or subsequent passage, give rise to the long form in the inoculated animal, with quite different symptoms (*cf.* figs. 3 and 4: fig. 3 photographed from a smear of spinal cord of Rat 66 *b*, and fig. 4 from a blood film of Rat 69, which was inoculated from Rat 66 *b*).

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* See Note on methods.

5. *Pathological Anatomy.*

The examination of these two series of rats has shown that the pathological effects are fairly uniform in each of the two types of cases.

In those animals in which paralysis occurred, the principal lesions were found in the spinal cord. There was invariably congestion of the vessels, and in eight cases well marked hæmorrhages around and in the substance of the cord. There was the characteristic exudation around the blood-vessels of mononuclear cells, and of plasma cells, such as Dr. Mott has described in the brain in cases of Sleeping Sickness; and in all the cases a varying amount of glia proliferation (*vide* figs. 1 and 2). The nerve-cells in the affected areas of the cord were degenerated in varying degrees up to the disappearance of the processes, and even of the nucleus with vacuolation of the protoplasm.

The brain showed much less change, and only in the chronic cases was any exudation found around the vessels. Small hæmorrhages in the meninges were seen in five cases.

Trypanosomata, mostly in stages of degeneration, were found scattered through the substance of the cord and brain in varying numbers; none were found in the vessels, which were generally distended with blood.

The other organs showed very little change; they were anæmic and firm; the spleen was not enlarged in the chronic cases with marked paralysis, and was only slightly enlarged in the transitional cases. There was no enlargement of the superficial or deep lymphatic glands, and the eyes and genitals were normal.

In the cases in which the trypanosomata were plenteous in the blood, and which had no paralytic symptoms (the Gambia Fever type), the brains and cords were very congested, and the blood in the distended vessels contained large numbers of trypanosomata. None were seen in the substance of the brain or cord. In six cases small hæmorrhages were found on the meninges. There was no degeneration of the brains or cords.

The superficial lymphatic glands were often enlarged. The abdominal glands were always enlarged, and some of both superficial and deep glands were reddish to brown in colour. The enlarged glands showed changes of a hyperplastic nature, and the reddish ones showed sinus formation, extreme congestion, and often hæmorrhages.

The lungs were always very congested, with sometimes subserous hæmorrhages in the lower lobes. Small hæmorrhages were found in 61 cases in the pleuræ.

The livers were always extremely congested, and were very soft in texture, and showed extensive parenchymatous degeneration, and often fatty infiltration and degeneration. The vessels were very dilated and distended, and often showed proliferation of their endothelium.

The stomach in 13 cases was ulcerated; generally one small ulcer filled with clot was found, but sometimes as many as three or four. They were always found on the greater curvature. In two cases death occurred from perforation.

There was generally a diffused congestion of the lining membrane of the intestines, and on the peritoneal surface ecchymoses dotted about, either singly or in groups, were often found. The kidneys were congested, and had always macro- or micro-scopical hæmorrhages.

The spleen in every case was enormously enlarged, with great congestion of the pulp, and considerable hyperplasia of the follicles. There was generally proliferation of the endothelium of the distended vessels, and a great deal of phagocytosis of red blood corpuscles and trypanosomata.

The bone marrow (femur) was profoundly altered, having the appearance of red marrow. It was very cellular, with scarcely any fat, and there was always considerable phagocytosis, and many nucleated red corpuscles.

6. *Conclusions.*

From the above summary of 211 experiments, extending over a period of nearly three years, it will be obvious that the tentative deductions which I made in my Preliminary Note from the few experiments therein recorded, that Gambia Fever and Sleeping Sickness are two distinct diseases, cannot be maintained.

This extended series of experiments and observations go to show that each of these two strains of trypanosomata has produced two different effects in the same class of animals, under conditions of which we at present know nothing; and that these effects are alike for the two organisms; and that the trypanosomata found in these two types of disease are one and the same organism, modified by passage from man through monkeys to rats, and perhaps in the strains I have used, by transplantation into animals of, and in, another country.

NOTE ON METHODS.

With regard to the mention made in Section 4 of methods, the following, which has been used by the writer for some years, gives uniformly good and, as he believes, accurate results. The specimen is never allowed to dry, and there is no shrinkage of the cells, and the finest cytological details can be observed.

1. Expose a cover-glass to the vapour of

Osmic acid, 1 per cent.	1 c.c.
Glacial acetic acid	3—5 drops

for 2 minutes.

(This can be most conveniently effected by using a block-dish covered by a thin glass having a hole in it rather smaller than the cover-glass used.)

2. Place a drop of fresh blood in one corner of the cover-glass, and expose again to the vapour for 30 seconds.

3. Spread the film carefully, and expose again for 15 to 30 seconds to the vapour until the surface appears no longer moist. (The film will not be really dry, and can be easily smeared off the glass with the finger: a really dry film will be much lighter in colour, and cannot be rubbed off with the finger.)

4. Place the cover-glass in absolute alcohol for 10 minutes.

5. Place the cover-glass in a faintly rose-coloured solution of permanganate of potash for 1 minute. (Two or three drops of a 1-per-cent. solution to 50 c.c. of water.)

6. Wash in water for 5 minutes.

7. Stain in a modified Romanowsky's stain, made by mixing just before use—

Azur I, 1 per cent.	1 c.c.
Eosin, B.A., 1—1000	2 „
Water	8 „

for 15—30 minutes.

8. Wash.
9. Differentiate in orange-tannin, 30 seconds.
10. Wash well and drain.
11. Absolute alcohol for a few seconds.
12. Alcohol—xylol (in proportion of 2 : 3), two or three changes.
13. Xylol, and mount.

Instead of 7—13, any other method of staining can be used, according to what structures it is desired particularly to show.

DESCRIPTION OF PLATE.

Fig. 1.—Section of half of spinal cord of rat which had been paralysed for 2 months before death. ×40. Shows meningitis, the exudation around the vessels, and a large area of degeneration.

Fig. 2.—Longitudinal section of vessel in spinal cord of rat which had been paralysed for 6 weeks before death. ×220. Shows the cellular exudation around the vessel, and on the right an area of degeneration.

Fig. 3.—Trypanosomata from a mash of spinal cord of rat, from which cord fig. 1 was photographed. ×1000. Stumpy forms, with very short flagella, staining badly, with very little differentiation.

Fig. 4.—Trypanosomata in blood of rat which was inoculated with the mash of spinal cord from which figs. 1 and 3 were photographed. Long, well-differentiated, easily staining forms. This rat was not paralysed.

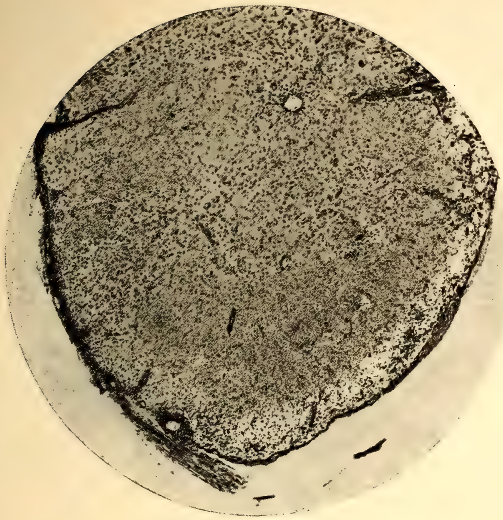

FIG. 1. ×40.

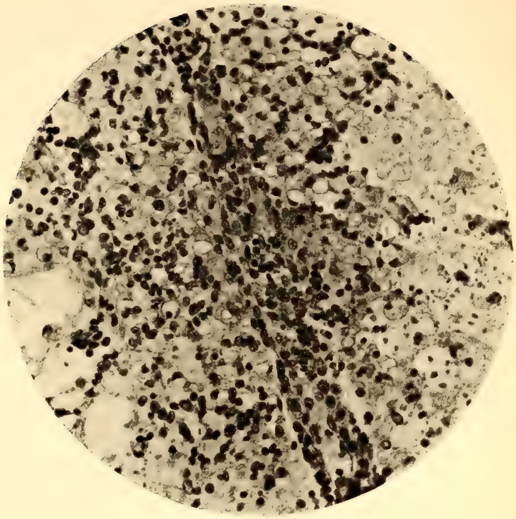

FIG. 2. ×220.

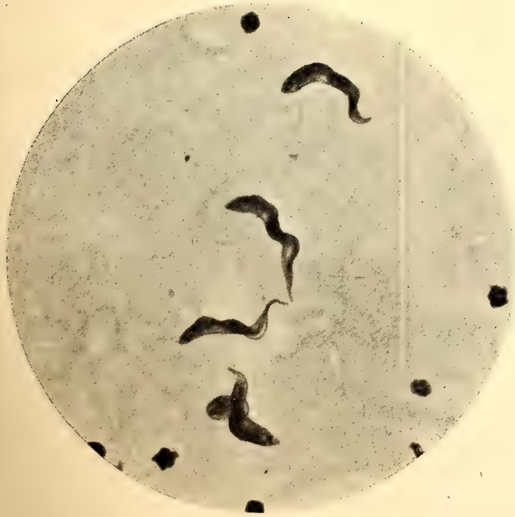

FIG. 3. ×1000.

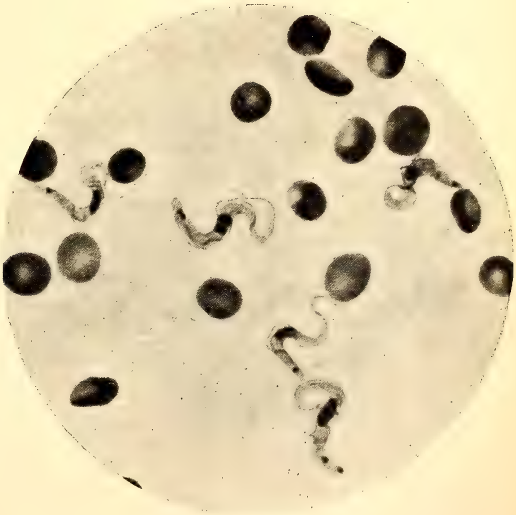

FIG. 4. ×1000.

OBITUARY NOTICES

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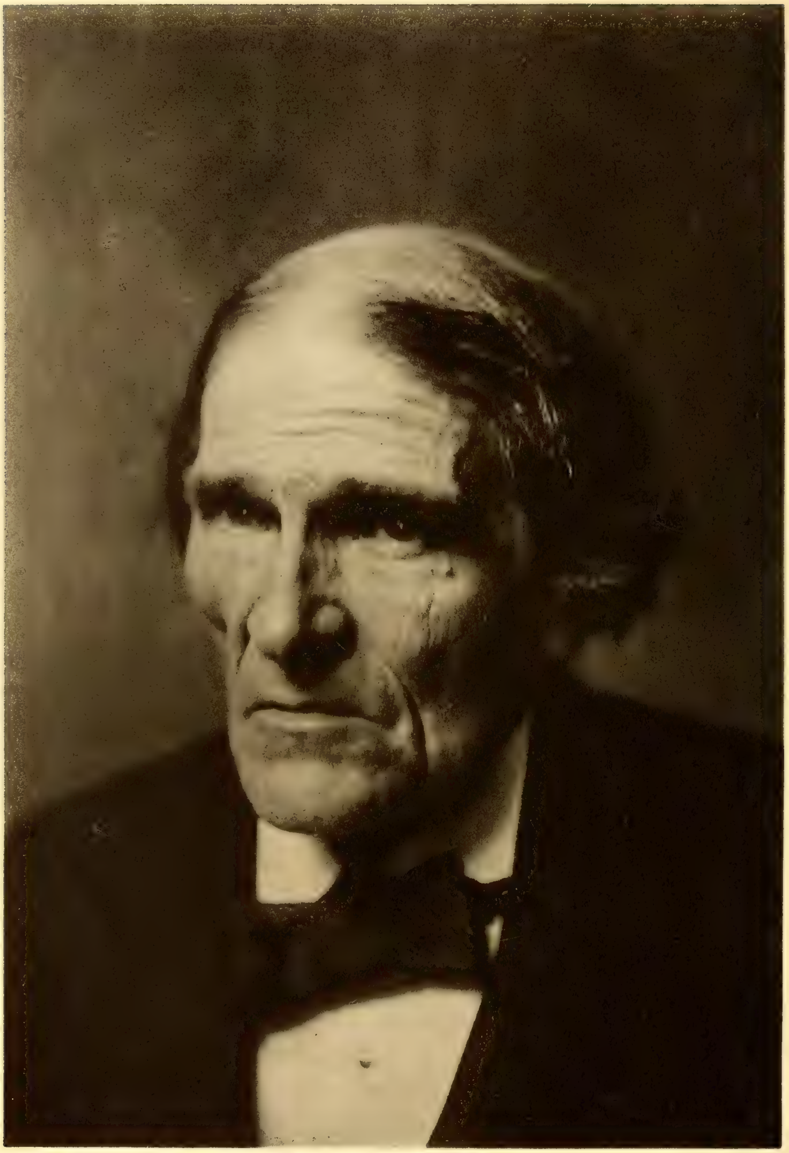

SIR JOHN BURDON SANDERSON.

On Intravascular Coagulation in Albinoes and Pigmented Animals, and on the Behaviour of the Nucleo-proteids of Testes in Solution in the Production of Intravascular Coagulation.

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The experiments described in this paper have been performed in the physiological laboratories of the University of London, and my best thanks are due to Dr. Augustus Waller, the Director, and to Mr. E. Legge Symes, the Demonstrator in Physiology, for the many facilities that were kindly placed at my service. Especially would I like to add my testimony to the value of the Dubois chloroform apparatus as a means of easily and safely administering chloroform to animals.

The cost of these experiments has been defrayed from a grant of the Government Grant Committee of the Royal Society.

PART I.—INTRODUCTION.

The work described in this paper was undertaken in order to ascertain the differences in relation to intravascular coagulation which distinguish the albino and pigmented races of a species.

There exists a considerable body of evidence tending to show that in certain qualities albinoes are less constitutionally vigorous than pigmented individuals (Darwin, 1899; Heusinger, 1846; Mudge, 1907; Twining, 1845; Prichard, 1855). Not a few cases of the converse kind are known, however, and are cited by Darwin (1899). Farabee (Farabee and Castle, 1903) has stated that albino negroes are taller and broader than their pigmented fellows, and we may interpret this difference as meaning some sort of physical superiority.

Quite recently we have had the experiments of Halliburton, Brodie, and Pickering (1896, 1897), which show that while the albinoes intravascularly injected by them with nucleo-proteids do not clot, their pigmented fellows do. Halliburton and Pickering (1897) further proved that the three synthesised colloids, A, B, and C of Grimaux, similarly clotted pigmented animals, but failed to do so with the albinoes used by them. Their experiments, while thus showing that albinoes may be more resistant than pigmented individuals, also apparently demonstrate the existence of a fundamental distinction between albinoes and pigmented individuals in their reaction towards injected nucleo-proteids.

But the results of the experiments described in this paper lead to a modification of such a conclusion, and show that two different sub-races of albinoes exist; one resembling pigmented animals, since its individuals clot upon the intravascular injection of nucleo-proteids, while the individuals of the other sub-race fail to clot.

Pickering (1897, 1899) further stated that the Norway hare in its winter coat reacts like an albino, but in its summer coat like a pigmented individual. The Norway hare, however, is not a complete albino when in its winter coat, for it has a patch of colour on its nose, at the tips of the ear pinnæ and the extremity of the tail, as well as possessing black eyes. The Himalayan domestic breed of rabbits, with which I have experimented, are similar to the Norway hare while in its winter coat, but they have pink and not black eyes. They are, therefore, not wholly comparable to the Norway hare. I find the Himalayan rabbits react like true albinoes.

PART II.—THE RELATION OF ALBINISM AND PIGMENTATION TO INJECTED NUCLEO-PROTEIDS.

(A) *Albinoes Injected with Nucleo-proteid derived from Pigmented Animals. Table II.*

The first question I investigated was whether albinoes invariably failed to clot when injected with a nucleo-proteid derived—like that used by Halliburton and Brodie—from pigmented animals. I injected (Table II) 43 albinoes, of which five were rats and the rest rabbits, with such a nucleo-proteid. I also injected eight of the partial albinoes known as Himalayan rabbits (p. 107 and Table V). Nine different solutions,* *i.e.*, B, H, D, I, J, K, N, O, and T, were used, most of them while freshly made (on day of preparation), but some both when freshly prepared and at stated intervals of two, five, seven, or more days after preparation, exclusive of the day on which they were made. The solutions were kept in an ice safe during these periods, and their physical characters, such as colour, odour, alkalinity, and viscosity were normal on each occasion of using. The results are shown in a tabulated form in Table II for the pure albinoes, and in Table V for the Himalayan rabbits.

The majority of pure albinoes manifested more or less extensive intravascular coagulation, the general intensity of which is shown in the sixth column of the table. In four albino rabbits, the blood absolutely failed to clot (experiments 26, 53, 71, and 173); while three gave a qualified failure of such a kind that we may, I think, regard it as of the same nature as the absolute failures (experiments 29, 30, and 72). In the case of the four absolute failures, as in all others, the solution was injected by one of the two methods described on p. 110 until death resulted, and an immediate *post-mortem* examination failed to reveal any clots anywhere, though I very carefully examined all the smallest pulmonary vessels that could be seen with the unaided eye, in addition to the main systemic and portal veins. In the three qualified cases, no clots comparable to those produced in a typical intravascular coagulation were found; for these latter clots in a typical case are strands of the form of the containing vessels and are red in colour. The clots which were found in these qualified cases were not red, but colourless, and they were not of the form of the containing vessel, but mere flocculi or flecks. Moreover, they never occurred in the veins, but always in the cavity of the right ventricle, and in two instances attached to the chordæ tendineæ, and in the other lying in the apex.

Three other qualified cases (experiments 6, 65, and 70) also occurred, but these, I think, must be regarded rather as very limited coagulations than as failures, because the clots were red. In experiment 70, the clot was red and very small, and it was attached to a few of the chordæ tendineæ where they joined the tricuspid valve. In experiments 6 and 65 no clots occurred anywhere, except that in the former case a very small red one was found in the postcaval vein just before it entered the heart; while in the latter case, in addition to a few colourless fibrin specks in the right ventricle, there was a semi-liquid red clot about 2 inches long formed in the postcaval vein just posterior to the liver.

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* As to mode of preparation of these solutions, see p. 122.

(B) *Albinoes Injected with Nucleo-proteid derived from Albinoes. Tables I and V.*

I next ascertained whether any failures of coagulation occurred when albinoes are injected with a nucleo-proteid derived from albino animals. As is shown in Table I, 24 albinoes were injected with albino nucleo-proteid, and of this number four were rats and the remainder rabbits. Two Himalayan rabbits were also similarly injected (Table V), but these are considered later (p. 107). Seven different solutions were used, *i.e.*, A, C, F, L, M, P, and S (p. 122). In most of these experiments the solution was freshly made, but in a few of them it was two or seven days old. In no case was there any condition which may be termed an absolute failure of coagulation. In experiment 140 there is the nearest approach to a qualified failure, for the only definite clotting which occurred was in the right ventricle, where a small, colourless, fibrin flocculus was formed. Careful examination of the smallest pulmonary vessels, *i.e.*, those just visible to the naked eye, showed, however, that the blood appeared to be viscous and forming a kind of liquid clot. It was impossible to decide definitely, owing to the smallness of the vessels. Certainly, in the vessels large enough to allow of a definite observation, the blood was neither clotted nor viscous. Experiment 165 helps us in a measure to interpret the results of the experiment just described. In this rabbit a few colourless fibrin clots were found in the right ventricle, as in rabbit 140, but, in addition, there were also a few red clots in the smaller branches of the pulmonary vessels, though the main vessels contained none. In experiment 80, the pulmonary vessels contained no clots, neither did any of the systemic or portal veins, but in the right ventricle there was a mixture, not intermingled, of colourless fibrin clots with the ordinary red clots.

These experiments justify the conclusion that when albinoes are injected with nucleo-proteid derived from albino animals, no absolute failure of coagulation occurs, such as happens in a certain percentage of cases when they are injected with nucleo-proteids derived from pigmented animals.

Doubtless, in the case of experiments 80 and 165, a much more extensive coagulation would have resulted had larger doses been injected; and the fact that coagulation occurred at all, with the formation of the ordinary red clots, with the minute doses used, is evidence that the animals were susceptible to the coagulative properties of the nucleo-proteid, and possibly the same consideration holds for experiment 140; the doubtfully viscous nature of the blood may have become a definite coagulation had a larger dose been injected. But even if we assume that in this rabbit the pulmonary blood in the smallest vessels was not viscous, the case still remains one of qualified and not of absolute failure; for the right ventricle contained colourless fibrin clots.

Thus, when albino rabbits are injected with "pigmented" nucleo-proteid, about 9 per cent. of absolute failures occur, and also about 7 per cent. of qualified failures. But when they are injected with "albino" nucleo-proteid no absolute failures occur, and it is very doubtful if any qualified ones do.

Partial Albinism (Himalayan Rabbits). Table V.

The characters of Himalayan rabbits are described on p. 104. The only feature concerning them which it is necessary to note is that, with the exception of the pink eyes, they externally resemble the winter condition of the Norway hare, in which Pickering failed to obtain intravascular coagulation. I injected 10 of these rabbits, eight with "pigmented" nucleo-proteid and two with "albino" nucleo-proteid (Table V). With respect to the eight rabbits, in experiment 28 there was an absolute failure of coagulation, and in experiment 8 a qualified failure, only a few colourless fibrin specks being detected in the right ventricle. In the remaining six cases, coagulation, though limited, was produced. Thus about 12 per cent. each of absolute and qualified failures occur.

The two which were injected with "albino" nucleo-proteid both clotted in a pronounced fashion, though the dose injected in one case (Table V, experiment 79) was very small. The number of experiments is too few to enable one to positively state that none of them when thus injected fail to clot. But if this should subsequently prove to be the case, and remembering that a certain percentage of them fail to clot when injected with "pigmented" nucleo-proteid, it is evident that Himalayan rabbits behave like pure albinoes.

The Cause of Failure to Coagulate is not due to the Solutions.

In those cases, both with the complete and partial albinoes, where coagulation failed to occur, the cause of failure is due to inherent qualities of the individuals and not to the solution or to environmental changes. The failures have occurred with both fresh (experiments 26 and 173) and kept (experiments 53 and 71) solutions. Moreover, fresh solutions may cause coagulation in some rabbits (experiments 24, 25, 27, 171, 172, and 174) and the same solution may yet fail in others (experiments 26 and 173). Smaller doses may suffice to clot in some cases (experiments 37, 57, 68), and larger, or sometimes very large doses of even the same solution fail in others (experiments 30 and 71). In all cases the solution is injected until death results. It is obvious that the failure to coagulate is an inherent quality of the organism and is independent of any possible changing conditions of the solution.

(C) *Pigmented Animals Injected with Nucleo-proteid derived from Albinoes. Table III.*

I next ascertained if when pigmented animals are injected with "albino" nucleo-proteid any failure of coagulation would occur, such as is the case when albinoes are injected with "pigmented" nucleo-proteid. Twenty-nine experiments under these conditions were performed and no failures of any kind occurred. There are, however, three instances, *i.e.*, experiments 136, 137, and 163, where the coagulation is very limited. In the first of these only one small clot in one of the smallest pulmonary vessels was found, though the shed blood clotted in 25 seconds. In the other two cases only a few clots could be found in the smallest pulmonary vessels. In experiment 92 the smallest pulmonary vessels contained thin axillary threads of clot, and there was a red clot in the apex of the right ventricle, but none elsewhere. In experiment 89, the coagulation, though very limited in extent, was more pronounced, for the right auricle and ventricle were full of red clot and the left ventricle contained some colourless fibrin clots, but none of the veins contained any. In case of the limited coagulation occurring in the rabbits (136 and 137) just described, it is to be noted that death occurred when a very minute dose only was injected. It is, therefore, probable that had not death occurred when it did, a larger injection would have caused more extensive coagulation.

In experiments 145, 151, 162, and 169, the chief object was to ascertain the degree of variation in the activity of the solution with increased age, and as my solution was limited in quantity, I could not with these particular individuals carry the injection far enough to produce either coagulation or death, owing to a marked reduction in the strength of the solution or to idiosyncrasy of the individuals. In experiments 145 and 162 the animals were idiosyncratic to a marked degree. In the four experiments mentioned, the animals were not therefore killed by the injections, but doubtless had it been possible to carry them far enough, coagulation would have occurred. In experiment 170, the animal died as the result of the injection, and with the typical symptoms of intravascular coagulation, but owing to the lateness of the hour, a *post-mortem* examination could not be made. These experiments do not, therefore, invalidate the conclusion that failure to clot does not occur when pigmented animals are injected with albino nucleo-proteids.

(D) *Pigmented Animals Injected with Nucleo-proteids derived from Pigmented Animals. Table IV.*

Table IV shows the result of injecting 63 pigmented rabbits with "pigmented" nucleo-proteid. No failure, absolute or qualified, occurred. One or two explanations are necessary. In experiment 34, the blood in the vessels escaped coagulation, but a large red clot was present in the right ventricle; the clotting was therefore of the typical nature, though very limited in extent. In experiment 157, the solution was used on the second day after its preparation, and a comparison of experiments 158, 159, and 160, where the solution was of the same age, with experiments 152 and 153, where it was used fresh, showed that a small fall in activity had occurred. But in experiment 157, a very large dose was injected without death resulting. I did not carry the injection further, because my solution was running low. Doubtless, had the injection been carried far enough, coagulation could have been induced; for it was not an infrequent phenomenon of these experiments that animals possessing an idiosyncratic resistance to injected nucleo-proteids now and then appeared and required abnormally large doses before coagulation could be brought about. Experiments 168 (Table IV), 54, 71, 73, and 74 (Table II) were with rabbits of this kind. In some instances, as in experiments 169, 170 (Table III), 75, and 76 (Table II), the large dose required was due to a falling-off in the activity of the solution through age; but such cases can be distinguished from the idiosyncratic ones by the fact that large doses were uniformly required in all the experiments in which the particular solution of a certain age was used. And in the experiment now under consideration, *i.e.*, 157 (Table IV), there is no doubt that the failure to kill is due to idiosyncrasy and that a sufficiently large dose would have produced coagulation. For larger doses than 16 c.c. per kilogramme of body weight have been necessary to produce such a result, as in experiments 1, 74, and 170, where 21 c.c., 23 c.c., and 20 c.c. respectively were required to clot. It may, therefore, be assumed that experiment 157 does not invalidate the conclusion that pigmented animals are always clotted upon the injection of a nucleo-proteid derived from pigmented animals.

With respect to experiments 166, 167, and 168 (Table IV), the injection resulted in death, with the usual symptoms accompanying death from intravascular coagulation, but I had not time to make a *post-mortem* examination in these cases. These experiments, in fact, were made for another purpose, *i.e.*, to ascertain the variation in strength of the solution with age.

PART III.

(A) *Relative Resistance of Albinoes and Pigmented Animals, as Measured by the Mean Quantity Injected Required to Produce Intravascular Coagulation. Tables I to V.*

In all my experiments the quantity of nucleo-proteid injected was measured and the body weight of the animal determined before the experiment commenced; with but few exceptions the animals were obtained some days before the experiments were made and were kept under uniform conditions with regard to time of feeding; so that differences in body weight were not due to relative differences of time between feeding and weighing. All the animals injected with Solution I, *i.e.*, 14 albinoes and 11 pigmented rabbits, were kept under the same conditions for an interval of six weeks.

The experiments were divided into two series. In the first, the injections were continuous though slow, and were continued until death resulted, the dose required and the time occupied being noted; the mean time is approximately 1 minute 6 seconds for every cubic centimetre injected. This method of injection I shall speak of as Method A. In the second series, very small doses, usually 0·1 c.c., were injected every two minutes until death resulted, and the total quantity injected then noted. As a rule death occurred at from 50 to 60 seconds after the lethal quantity had been injected. This method I shall name Method B, and it is sometimes alluded to as the method of incremental doses. In all cases the injection was made through one of the marginal ear veins, by a syringe capable of containing 11 c.c. of solution. The results of these experiments and the experimental data are given in a tabulated form in Tables I to V, pp. 125—129.

In making the comparisons between the mean dose required to kill pigmented and albino animals, in either of the two series (*i.e.*, in the series of injections of albino and pigmented animals with "albino" nucleo-proteid and in those with "pigmented" nucleo-proteid), we can either take the results derived from each separate solution (group comparisons), or they can be compared in the aggregate (final comparison). Neither one of these two modes of comparison is preferable to the other; since while in the former the factors in each group are homogeneous, it follows that in the latter each pair of groups (that for the albinoes and pigmented individuals) are also homogeneous. The sequel will show that the conclusion is the same by either method. All group comparisons must be made not only with animals injected with the same solution, but with the same solution of the same age, because the strength of the solution varies with its age (*infra*, p. 119). Below, in Table VI, I give the result in a tabulated form, of obtaining the mean dose required to kill albinoes and pigmented animals respectively with each of the solutions used (group mean doses). The means are calculated from the data given in Tables I to V, pp. 125—129.

Table VI.

Solution.	Age of solution.[*]	Pigmented animals.		Albinoes.	
		Mean dose per kilogramme of body weight.	Experiments.	Mean dose per kilogramme of body weight.	Experiments.
Series 1.—Continuous but slow injections until death results (Method A).					
I	0	3·68	19—23	4·4	24—27
	5	1·18	32, 34, 36	1·41	29, 30, 31, 33, 35, 37
	7	0·76	43	4·28	40—42
J	2	0·52	57	3·21 or 7·37[†]	53, *54*, 56
Series 2.—Incremental doses, usually of 0·1 c c. at 2′ intervals until death results (Method B).					
K	0	0·484 or 0·92[†]	59—64, *63*	0·705 or 1·83[†]	65—*67*
L	0	0·345	83, 84	0·458	77, 78, 80—82
M	0	0·26	86, 89, 90, 92, 94, 96, 98	0·38	85, 87, 88, 91, 93, 95, 97, 99, 100
N	0	0·206	102, 104, 106	0·33	101, 103, 105, 107
O	0	0·22	108, 110, 113, 114	0·286	109, 111, 115
	2	0·38	116—120, 122	0·58	121
P	0	0·138	136—139	0·235 or 0·437[†]	140—*141*
S	2	0·45 or 2·40[†]	161, *162*, 163	1·0	164, 165
T	0	0·41 or 1·75[†]	171, *172*	0·42 or 2·1[†]	173, *174*

[*] The figures under column "Age of solution" have the following meaning:—0 = solution used on day of preparation; 2, 5, 7, etc. = solution used on the 2nd, 5th or 7th, etc., day after that of preparation and exclusive of it.

[†] The alternative figures are rendered necessary by the appearance in certain experiments (indicated by their reference number being in italics) of individuals of marked idiosyncrasy. The lower figure is the result of excluding these experiments from the calculation.

In arriving at the results tabulated above (Table VI, †) I have, in the case of certain experiments where the influence of idiosyncrasy manifested itself, given two alternative mean results, in one of which the idiosyncratic animals have been excluded and in the other included. But whichever of the two means is accepted, as the alternative ratios given show, the general result is the same, *i.e.*, the albino is markedly more resistant than the pigmented animal.

An inspection of the figures in Table VI shows at once that in each group of experiments, and with each solution used, whatever its age, the albinoes require a larger mean dose per kilogramme of body weight in order to produce death by intravascular coagulation. If the mean of all the means in the above table is ascertained, it is found that for the pigmented animals it is 0·69 c.c. or 0·98 c.c. per kilogramme of body weight, and for the albinoes it is 1·36 c.c. or 1·91 c.c. The alternative figures depend upon whether the lower or higher mean under Solutions J, K, P, and T for the albinoes, and K, S, and T for the pigmented animals is included in the calculations.

The albinoes thus appear to be about twice as resistant to the coagulating influence of nucleo-proteids than do the pigmented animals, if the idiosyncratic individuals are excluded, or rather less if they are included in the calculations. And, in whichever way we deal with the figures, the same result comes out. For if, instead of taking the mean of the means included in both Series 1 and 2, we ascertain the mean of the means in each series separately, we reach the following figures:—The mean of all the means in Series 1 is for pigmented animals 1·53 c.c. and for albinoes 3·32 or 4·36 c.c. per kilogramme of body weight. The mean of the means in Series 2 is for pigmented rabbits 0·320 or 0·735 c.c., and for albinoes 0·488 or 0·822 c.c. per kilogramme of body weight.

Thus by the Method A of injecting, the relative resisting power of albinoes is about double that of the pigmented animals, the ratio being 2·16 or 2·8, and by the Method B it is less than double, the ratio being 1·52 in favour of the albino. But in the alternative results, under Method B, *i.e.*, those in which the idiosyncratic individuals are included, the pigmented and albino individuals are about equally resistant, the ratio being 1·1 in favour of the albino individuals. This result is apparent and not real, and is due to the markedly idiosyncratic pigmented individual, 162, under Solution S and, as it has been pointed out on p. 115, Part III (B), there can be no doubt that it should be rejected. If the result of the S solution be rejected altogether on both sides—for both the pigmented and albino series—the result is a mean dose for the pigmented animals of 0·527 instead of 0·868, and for the albinoes of 0·80 instead of 0·822; the ratio thus becomes 1·5 in favour of the albinoes. This ratio is the same as that for the whole series under Method B, when all the idiosyncratic animals are excluded.

The difference in the ratios of Series 1 and 2, *i.e.*, 2·16 for the former and 1·52 for the latter, is partly due to the fact that Method A of injection

(Series 1) is not so refined as Method B (Series 2), and partly to the fact that in Series 1 a certain number of experiments in which the solution was used on the second, fifth, and seventh days after preparation are included in the mean results, while fewer of such experiments are included in the second series; the reason for the operation of this latter influence is given *infra*, p. 113. That the ratio of the two series should be in the same direction is corroborative proof of the truth of each.

One possible source of error, arising from a want of complete homogeneity, remains to be considered. It will be seen from an inspection of Tables II and IV, or of Table VI, that the I solution, when of seven days' standing after its preparation, was injected into only one pigmented rabbit (43) but into three albinoes (40 to 42), and that the dose for the pigmented animal is very small. It is possible that had another two pigmented rabbits been used, the mean dose of the solution of this age would have been raised for the pigmented animals. The same consideration holds for the J solution on the second day after its preparation; for only one experiment was performed on a pigmented individual (57), and two on albinoes (53 and 56). But it is obvious from a review of the means of these two solutions at other ages and of the other solutions, that had the number of pigmented and albino individuals been more nearly equal in these particular experiments, the mean result would still have been higher for the albino and the ratio would have been near that arrived at. That it would have been so is rendered all the more certain by the fact that the ratio of resistance of albino and pigmented animals is increased as the age of the solution increases; this fact alone explains the large difference between the albino and pigmented races in these two experiments, *i.e.*, those of the I and J solutions of seven and of two days' standing.

The greater resistance of the albinoes is graphically shown in fig. 1, where the upper line, *i.e.*, line of larger dose is the albino line. *Cp.* p. 117.

(B) *The Relative Resistance of Albinoes and Pigmented Animals to Nucleo-Proteids derived from the Two Alternative Sources. Tables VI and VII.*

The results arrived at above show that albinoes are more resistant to injections of nucleo-proteids than are pigmented individuals, and that this is the case when the nucleo-proteid is derived from either pigmented or albino individuals. The experiments, however, enable us to ascertain whether albinoes and pigmented animals are more resistant or less resistant to nucleo-proteids derived from their own source, or from the alternative source. For, by comparing the mean results (p. 115) of injecting albinoes with "albino" nucleo-proteid with those obtained by using "pigmented" nucleo-proteid, any difference which may exist in the reaction of albinoes towards nucleo-proteids derived from these two sources, can be ascertained. And the same fact can be similarly ascertained for the pigmented animals.

The mean doses required to kill albino and pigmented animals when both kinds of nucleo-proteids are used in each instance may be obtained by calculation from Tables I to IV, or more quickly from the mean results of Table VI, p. 111. Those experiments only in which the injections were made by the incremental or B method, can be utilised for this purpose, because no albinoes were injected by the A method with nucleo-proteids derived from albinoes. And also, with but one exception, only those experiments have been included in the calculations in which the solutions were used on the day of their preparation; the number of experiments in which older solutions were used are not sufficient to enable more comparisons to be made. Owing to the existence of a few individuals which manifested more or less marked idiosyncratic resistance, it is necessary to obtain, both for the albinoes and the pigmented animals, two mean results instead of one. One of these two results in each case will include the idiosyncratic animals and the other will not. The animals which are regarded as idiosyncratic are those in experiments 63 and 67, Sol. K; 141, Sol. P; 172 and 174, Sol. T; and 162, Sol. S; Tables I to IV and VI.

If we now ascertain the mean dose of the "albino" nucleo-proteid (Solutions L, M, P, and S) required to kill albino animals, it is 0·518 c.c. per kilogramme of body weight, if the idiosyncratic animal 63 is excluded, and 0·575 if included (Table VII). If we similarly determine the mean dose of nucleo-proteid derived from pigmented animals (Solutions K, N, O, T) necessary to kill albinoes, it is found to be 0·435 c.c. (excluding experiment 67), or 1·136 c.c. including it. Thus, by rejecting the idiosyncratic individuals, the mean dose of "pigmented" nucleo-proteid necessary to kill albinoes appears to be smaller than that obtained from albinoes. The albino, therefore, is more resistant to its own nucleo-proteids than to those obtained from pigmented animals.

The mean dose required to kill pigmented rabbits by the injection of "albino" nucleo-proteid is 0·298 c.c., or 1·08 c.c. per kilogramme of body weight; the great increase due to idiosyncrasy occurs under the S solution in relation to one animal only (162), and it must obviously be rejected. The mean dose of "pigmented" nucleo-proteid required to kill them is 0·330 c.c. if we reject the idiosyncrasies (experiments 63 and 172), and 0·777 c.c. if we include them. Thus, disregarding the idiosyncrasies, the pigmented animals appear to require a larger mean dose of their own nucleo-proteid to clot their blood than they do of that obtained from albinoes. They are thus more resistant to "pigmented" nucleo-proteid than they are to "albino" nucleo-proteid.

If the idiosyncratic animals are included, however, the results are in both cases reversed. The rejected experiments are 63 and 172 (Table IV), 162 (Table III), and 141 (Table I). An inspection of the figures given in the tables will show that these individuals were markedly idiosyncratic, for rabbits Nos. 141, 63, 172, and 162 were respectively three, six, eight, and twelve times more resistant than the average of their fellows injected with the same solution at the same time. They clearly stand above their fellows as marked mutations, and must therefore be excluded in any statement of a mean result.

Stating the results described above in a generalised form, they may be expressed as follows: that both albino and pigmented individuals are more resistant to nucleo-proteids obtained from individuals of their own race than they are to those obtained from the alternative source.

In the following table (VII) the group mean doses extracted from Table VI are arranged to illustrate the results just stated; and, in addition, for the purposes of a later discussion, there is also included the weight of the testes employed in the preparation of the different solutions used. The idiosyncratic animals are not included.

Table VII.—To Illustrate, in the Case of both Albino and Pigmented Animals, the Relative Resistance to "Albino" and "Pigmented" Nucleo-proteids.

Solution used.	Weight of testis.	Mean dose when injected with "albino" nucleo-proteid.	Solution used.	Weight of testis.	Mean dose when injected with "pigmented" nucleo-proteid.
		Albinoes.			
	grammes.	c.c.		grammes.	c.c.
P	1·0	0·235	N	0·6	0·330
L	3·2	0·458	O	2·9	0·286
M	4·2	0·380	T	5·2	0·420
S	5·0	1·000	K	5·5	0·705
Mean weight of gland	3·35	Mean of the means 0·518	Mean weight of gland	3·7	Mean of the means 0·435
		Pigmented Individuals.			
P	1·0	0·138	N	0·6	0·206
L	3·2	0·345	O	2·9	0·220
M	4·2	0·260	T	5·2	0·410
S	5·0	0·450	K	5·5	0·484
Mean of the means 0·298			Mean of the means 0·330		

An inspection of the table will show that the conditions of the experiments were nearly, but not quite, equal. A comparison of the relative resistance of albinoes and pigmented animals towards nucleo-proteids derived from both albino and pigmented individuals has been made. It is obvious that the nucleo-proteid solutions must be equal in every respect, except in their origin, if the comparison is to be fair. But the mean weight of the glands used in the preparation of the "pigmented" nucleo-proteid is 3·7 grammes, while that for the glands used in the preparation of the "albino" nucleo-proteid is 3·35 grammes. If there should exist any difference in the activity of solutions made from spermatic glands at different stages of maturity, it is obvious that, under the conditions of the experiments, the conclusions stated above would possibly require slight modification. This possibility is discussed (Part IV, A, p. 118). It may be stated, however, that such a modification is probably not needed.

Part IV.

(A) *Variation in the Activity or Quantity of Nucleo-proteids derived from Testes in different Stages of Maturity.*

I was led to investigate, through the suggestion of other facts which I had previously ascertained, and which are described in Section B, what influence weight of gland (and presumably, therefore, degree of maturity) had upon the activity of the solutions made from them. In Table VII are given the weights of the testes (p. 115) from which the solutions used were made, and it will be seen that the mean weight of all the glands used in the preparation of the "albino" solutions was 3·35 grammes, and that of glands used in the preparation of the "pigmented" solutions was 3·7 grammes. If, then, the heavier glands should be either more or less active in the production of nucleo-proteids than the lighter ones of the younger individuals, it is obvious that this slight difference in the mean weight of the glands used from the albino and pigmented animals respectively, might introduce a disturbing factor, and the arrangement of the experimental data available (as in Table VII) in a form to show the relative resisting powers of albinoes and pigmented individuals towards the two classes of nucleo-proteids, might give apparent and not real results.

Accordingly, the experimental data were arranged in a way to show what was the influence of the weight of gland upon the activity of the solutions of nucleo-proteids. I had no data as to the age of the rabbits from which the glands were extracted, but as every gland used had been carefully weighed, and the colour of the individuals from which they were obtained had been recorded, I knew the weight, origin, and number of glands used in the preparation of each of the solutions (p. 122). To an approximate degree of accuracy, it may be assumed that weight of gland will coincide with the proportional age of the animal from which it was extracted.

If the results (only those obtained from the Method B of injection are used) are plotted out, as in fig. 1, in such a way that the abscissæ indicate the weight of the glands, and the ordinates express the measure of the mean dose of the solutions prepared from glands of different weights required to clot the blood of either albinoes or pigmented individuals, one fact stands out prominently. An examination of the figure shows that on the whole there is a fall in strength of the solution as the gland increases in weight (age), since a solution made from a heavy gland requires to be used in a larger mean dose per kilogramme of body weight to produce intravascular clotting than does one made from a lighter (younger) gland. The curves, while on the whole gradually trending upwards, show, however, that the fall in strength is not one without any fluctuations, and the nature of these will be considered later.

The two curves in fig. 1 very clearly show the greater resisting power of the albinoes towards injected nucleo-proteids than that of the pigmented individuals, for the upper curve represents the former, and the lower the latter, and practically through the whole of its range, the " albino line " is higher than the "pigmented line." The solutions are the same for both varieties of rabbits throughout. The two curves are constructed from all the results; that is, the mean doses of the solutions of both "pigmented" and "albino" nucleo-proteids are used together. In the figure, the solutions are indicated by their reference letters, and the small letter to the upper and right side of them, shows whether it is a solution of a "pigmented" or "albino" nucleo-proteid.

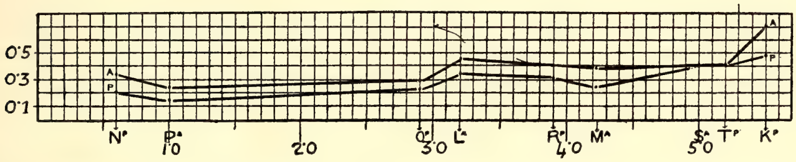

Fig. 1.—Showing the progressive fall in activity of solutions of nucleo-proteids derived from testes of increasing weight (age). The upper line is for albino animals, and the lower line for pigmented individuals. The nature of the solution used (p. 122) is indicated by the large letters, and its origin, *i.e.*, whether from glands of pigmented or albino individuals, is shown by the small index letters P and A respectively. The abscissæ show the weight of the glands in grammes, and the ordinates the amount of injection in cubic centimetres per kilogramme of body weight necessary to kill by intravascular coagulation.

In fig. 2, however, the series of solutions are separated, so that four curves are shown, two each for the "albino" and "pigmented" solutions. The existence of two curves for each solution is due to the fact that the two kinds of solution were injected into both albinoes and pigmented individuals. The higher resisting power of the albino is again well shown and towards both kinds of nucleo-proteids, for the upper line in the two pairs of curves is the "albino" one. And it is obvious that in the general trend, *i.e.*, increasing loss of activity with increasing maturity of gland, the testes of both the albino and the pigmented animal behave much in the same way.

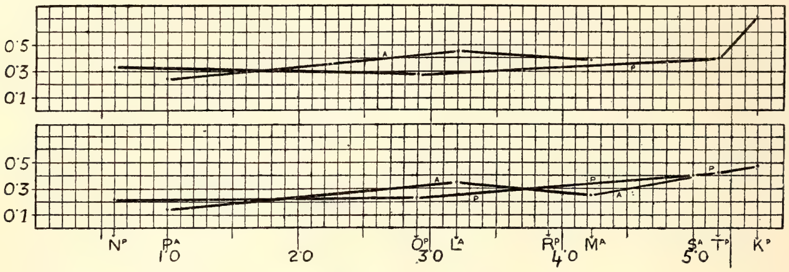

Fig. 2.—The combined results shown in fig. 1 are here separated. In the upper figure the albino individuals only are considered, and in the lower one the pigmented individuals only. The existence of two curves for both albinoes and pigmented animals is due to the fact that both races were each injected with "albino" and "pigmented" nucleo-proteid. The "albino and pigmented solution" lines are indicated by A and P respectively. The other reference letters and numbers have the same significance as those in fig. 1.

But there is a difference of behaviour at different stages of maturity, and fig. 2 shows that the somewhat abrupt fall in strength at 3·2 grammes weight of testis in fig. 1 is due to the albino gland; but, on the other hand, the increase of strength at 1 gramme and at 4·2 grammes is mainly due also to the albino gland. At the 5 gramme weight stage of maturity the testes of albinoes and pigmented animals appear to be equal in this respect.

The same general trend of variation in activity is shown by solutions on the second day after their preparation, for, as far as the observations go, the maturer glands give less active solutions, and the same may be said of solutions used on the fifth day after their preparation.

Reverting to the conclusion stated on p. 115, that both albino and pigmented animals are more resistant to nucleo-proteids derived from glands of individuals of the same race, and less resistant to those obtained from the alternative source, it must be borne in mind that this is a mean result.

But an inspection of the curves in fig. 2 (upper and lower), shows that upon analysis this result apparently holds true for a part only of the range of gland-weight. If the conclusion were generally true, the curve for pigmented animals injected by "albino" nucleo-proteid should be lower throughout its range than for those injected by "pigmented" nucleo-proteid; and the curve for albino individuals injected with "pigmented" nucleo-proteid should also be lower throughout its range than that for those injected by "albino" nucleo-proteid. But this is not wholly the case. With the albino individuals (fig. 2, upper) the two curves are relatively as they should be throughout the greater part of their range, the lower one crossing the upper one towards the left. But this crossing obviously may be due to the absence of any data for "pigmented" testis weighing 1 gramme, and to that for "albino" testis weighing 0·6 gramme. The same considerations hold for the pigmented animals (fig. 2, lower); had data been available for "pigmented" testes of 1 gramme and 4·2 grammes, the "pigmented" curve may have been lowered at these two points, and they would then have followed a uniform course throughout their range, the "albino" line being the higher.

(B) *Variation in the Activity of Solutions of Nucleo-proteids with Increasing Age.*

In the experiments described in this paper, most of the injections were made on the same day that the solutions of nucleo-proteid were prepared, some of them two days later (so that the solution, inclusive of the day of its preparation, was then three days old), and some of them five days later (the solution then being six days old), and a few at still later periods. If we examine the results obtained by using the same solution on different days it is found that the mean dose per kilogramme of body weight required to clot varies with the age of the solution. There were seven different solutions, *i.e.*, I, K, O, P, R, S, and T, each of which were used on different days. Four of these, *i.e.*, R, S, T, and K, which were derived from heavier glands (*cp.* p. 122 and figs. 1 and 2), gave a continuous fall of strength with increasing period of keeping, but three of them, *i.e.*, I, O, and P, which were derived from lighter glands, gave different results (fig. 3).

The R, S, and T solutions were tested on the day of preparation and on the second and fifth days after, and the K solution on the day of preparation and on the second day after. All these solutions fell in strength with each successive period, but the S solution fell markedly on the fifth day. With respect to the T solution, experiment 172 has to be eliminated, owing to idiosyncrasy; how marked it is can be seen by comparison with experiment 171.

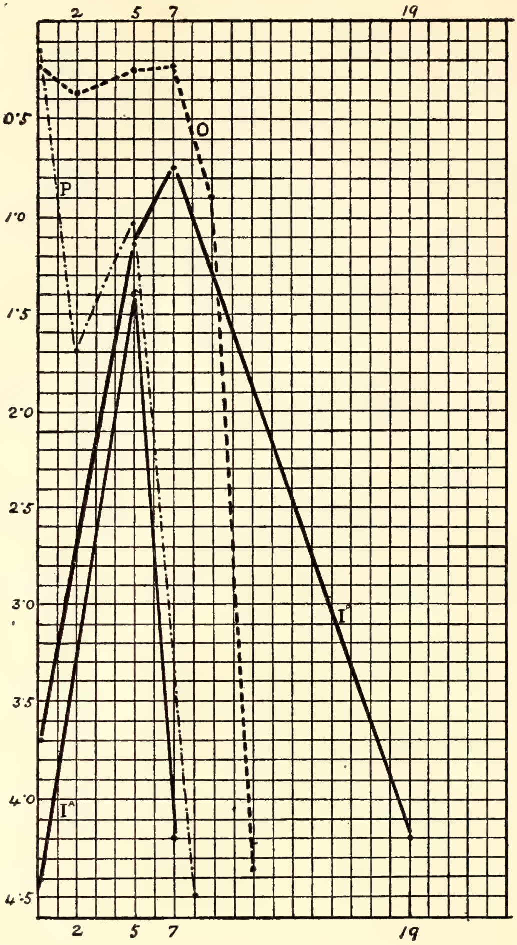

Fig. 3.—Curves showing the fluctuation in activity of solutions of nucleo-proteids with increasing period of keeping. The abscissæ show the age in days of the solutions, and the ordinates the amount of the injection in cubic centimetres of the solution per kilogramme of body weight to produce death by intravascular coagulation. The solutions used (*cp.* p. 122) are indicated by the letters attached to the curves. The curves marked I^A and I^P are for albinoes and pigmented individuals respectively. The curves for O and P are for pigmented individuals only.

The behaviour of Solutions I, O, and P is graphically represented in fig. 3. With Solution O, the mean dose required to clot on the day of its preparation was 0·24 c.c. per kilogramme of body weight; on the second day afterwards (third day old solution) the activity had fallen, and a mean dose of 0·38 c.c. per kilogramme of body weight was required to clot; on the fifth day after preparation the strength had increased to 0·252 c.c.; this increase continued until the seventh day of preparation, when it was slightly stronger, *i.e.*, 0·236 c.c., that is, nearly as active as on the day of its preparation; on the ninth day it had fallen in activity considerably, *i.e.*, to 0·9 c.c., and on the 11th day the loss of strength was very great, to 4·36 c.c. The same fall on the second day, with a subsequent rise in strength on the fifth, is shown in a yet more marked manner by the curve for Solution P.

In the case of the I solution, for which two curves are shown, one for the albino animals (I^A) and one for the pigmented animals (I^P), no examination of its strength on the second day was made. But for both pigmented and albino animals there is a very marked rise in activity on the fifth day after the preparation of the solution. In the case of the albinoes the strength of the solution falls on the seventh day, but rises for the pigmented animals; this difference may be due to the fact that only one pigmented rabbit was injected on the seventh day; but, bearing in mind the curve for Solution O, it is possible it may have still retained its present form if more experiments had been performed. On the 19th day the activity of the solution, when used on pigmented animals, fell very markedly. It is very probable that had Solution I been tested on the second day, that it would have shown, like Solutions O and P, a fall in activity. My experiments, however, at this period were not designed to investigate this problem, and I did not consequently test the solution on the second day.

SUMMARY OF CONCLUSIONS.

1. When albinoes are injected with a solution of nucleo-proteid derived from a pigmented animal, a certain number of them, about 9 per cent., absolutely fail to clot, while about 7 per cent. give a qualified clotting, the remainder giving a typical intravascular coagulation of more or less extensive development.

2. When albinoes are similarly injected with a solution of nucleo-proteid, but derived from albinoes, no absolute failure of coagulation occurs, and it is very doubtful if any qualified ones do. The great majority clot as distinctly as do pigmented individuals.

3. When pigmented rabbits are injected with solutions of nucleo-proteids, derived from albinoes or with those derived from pigmented individuals, no failures of coagulation occur.

4. The Himalayan rabbit, in respect of its reaction to injected nucleo-proteids, behaves like the complete albino. This rabbit, though resembling the Norway hare in its winter coat, in which condition Pickering failed to obtain intravascular coagulation, differs from it in having pink

(unpigmented) instead of pigmented eyes and in never becoming periodically wholly pigmented. It cannot, therefore, be used as corroborative evidence of Pickering's conclusion with respect to the Norway hare.

5. That failures to coagulate, when they occur, are due to inherent qualities of the individuals and not to weakening in the activity of the solutions used.

6. Albinoes require a larger mean dose per kilogramme of body weight of injected nucleo-proteid to cause death by intravascular coagulation than do pigmented animals, the relative resisting powers of the pigmented and albino individuals being as 1 to 1·5 respectively.

7. Both albino and pigmented individuals are more resistant to nucleo-proteids, obtained from individuals of their own race, than they are to those obtained from the alternative source.

8. The activity of a solution of nucleo-proteid, prepared from spermatic glands, decreases (but not quite uniformly) as the maturity (weight) of the gland increases.

9. Solutions of nucleo-proteids, prepared from heavier (maturer) spermatic glands, undergo a progressive loss of activity with increasing period of keeping, *i.e.*, from 1 to 20 days. But solutions derived from lighter (immature) glands undergo a fluctuating variation in activity, falling off on the second day after preparation and rising again on the fifth to seventh, and thence exhibiting a progressive fall.

NATURE OF SOLUTIONS USED.

W.G. = weight of gland ; D. = dissolved in 1-per-cent. solution of anhydrous Na_2CO_3. The amount of solution used in each case is indicated in cubic centimetres. Unless otherwise stated, all the solutions were prepared by pressing the glands through finely meshed iron wire gauze into 1-per-cent. solution of Na_2CO_3.

Sol. A.—(1) Thymus glands from four albino rats, 6 weeks old, descended from albino parents. (2) Testes from albino rat of unknown parentage. (3) Thymus from albino rat, 8 weeks old, from black and white parents. W.G. = 4·2 grammes, D. in 65 c.c. = 1 gramme in 15·5 c.c.

Sol. B.—Thymus gland of a black and white calf. Portion of organ minced and ground in a mortar with an equal volume of sodium chloride. Separated the nucleo-proteid by addition of distilled water, and collected into 1-per-cent. solution of Na_2CO_3. Strength of solution was not ascertained, but was probably nearly saturated.

Sol. C.—Testes from albino rabbit. W.G. = 6 grammes, D. in 100 c.c. = 1 gramme in 15·5 c.c.

Sol. D.—Thymus glands from four young pigmented rabbits, with much unpigmented coat. Glands crushed in distilled water, macerated for 36 hours, and the nucleo-proteid then precipitated by 0·2-per-cent. acetic acid. Dissolved in 70 c.c. of 1-per-cent. solution of Na_2CO_3.

Sol. F.—Testes from two albino rats of albino parentage. W.G. = 3·6 grammes, D. in 50 c.c. = 1 gramme in 14 c.c.

Sol. G.—Testes from Himalayan rabbit. W.G. = 2·2 grammes, D. in 32 c.c. = 1 gramme in 14·5 c.c.

Sol. H.—Thymus glands from three black and one brown rabbit. Dissolved in 1-per-cent. solution of Na_2CO_3.

Sol. I.—Testes from five brown (grey), one black, one silver grey, one fawn and one brown and white rabbits. The glands from the five brown rabbits weighed 8 grammes, and those from the remaining four rabbits 19 grammes. Total W.G. = 27·2 grammes, D. in 423 c.c. = 1 gramme in 15·5 c.c.

Sol. J.—Testes from a black and white rabbit, a blackish-grey, and a brown one. W.G. = 11 grammes, D. in 170·5 c.c. = 1 gramme in 15·5 c.c.

Sol. K.—Two testes from a brown rabbit. W.G. = 5·5 grammes, D. in 85 c.c. = 1 gramme in 15·5 c.c.

Sol. L.—Testes from albino rabbit. W.G. = 3·2 grammes, D. in 49 c.c. = 1 gramme in 15·5 c.c.

Sol. M.—Testes from albino rabbit. W.G. = 4·2 grammes, D. in 65 c.c. = 1 gramme in 15·5 c.c.

Sol. N.—Testes from two grey (brown) rabbits. W.G. = 1·2 gramme, D in 18·6 c.c. = 1 gramme in 15·5 c.c.

Sol. O.—Testes from a grey (brown) rabbit. W.G. = 2·9 grammes, D. in 45 c.c. = 1 gramme in 15·5 c.c.

Sol. P.—Testes from an albino rabbit. W.G. = 1 gramme, D. in 15·5 c.c.

Sol. R.—Testes from one fawn and one grey rabbit. W.G. = 7·8 grammes, D. in 121 c.c. = 1 gramme in 15·5 c.c.

Sol. S.—Testes from Himalayan rabbit. W.G. = 5 grammes, D. in 77·5 c.c. = 1 gramme in 15·5 c.c.

Sol. T.—Testes from black rabbit. W.G. = 5·2 grammes, D. in 80·6 c.c. = 1 gramme in 15·5 c.c.

EXPLANATIONS OF THE ABBREVIATIONS USED IN THE TABLES I—V.

"Albino" solution and "pigmented" solution mean a solution of nucleo-proteid derived from albino and from pigmented animals respectively.

The capital letter in the column headed "Solution used" refers to the nature of the solution used. A description is to be found on p. 122. The small numeral at the top right-hand side of the capital letter indicates the age of the solution when used. Thus L^0 means that it was prepared and injected on the same day: and I^5 that it was injected on the fifth day after its preparation, exclusive of the day of preparation. The solutions were kept in an ice safe, gave no signs of putrefaction, and retained their physical characters at the time of using.

V.P. = Very pronounced. All the vessels examined; *i.e.*, right and left precavals, right and left external jugulars, the whole length of the postcaval (that lying in the thorax, and for brevity called "thoracic portion," and that lying in the abdominal cavity and called "abdominal portion" or "abdominal postcaval"), and the hepatic-portal veins, being filled solid with continuous axillary strands.

P. = Pronounced. The clot in the vessels either fills it solid, as above, or does not quite fill it, but in either instance is absent from the "thoracic postcaval," or from the portal vein, or from both.

F.P. = Fairly pronounced. The clot is axillary and continuous, but thin, and is absent from the "thoracic postcaval" or portal vein, or from both.

F. = Faint coagulation. The clot is a mere cotton-like thread, and is more or less continuous and axillary, but is absent from the "thoracic postcaval" and portal vein.

V.F. = Very faint coagulation. The chief feature of this condition is in its limitation, for the clot may be a mere cotton-like thread or fairly thick (nearly as thick as in P). The clot is limited, however, to one or other of the two jugulars and associated precavals, or is confined to any other one vein; and small clots may or may not be present in the chambers of the heart.

A.B. = Coagulation almost absent. Only a few *colourless* clots of fibrin specks or filaments are present in the ventricles of the heart, and usually attached to the chordæ tendineæ. There are no clots of any kind in any of the veins.

A.B′. = Like A.B., but the clot in the chambers of the heart is red (not colourless) and is fairly large, varying from a tenth of an inch to a clot which fills the chamber.

A. = Absence of coagulation absolute.

The above definitions of conditions are based upon actually occurring states, and are not therefore wholly arbitrary; but in a few of the experiments it is difficult to assign the observed state to any one of the above defined groups. Such instances are indicated in the tables by the interrogative sign.

No result = Means that as much of the solution that could be spared was injected, but was not sufficient to kill the animal. It was then killed by an overdose of chloroform.

No P.M. exam. = A *post-mortem* examination was not made. In the few cases in which the examination was omitted (owing to the lateness of the hour) the symptoms were typically those of death by intravasculation coagulation. The object of the experiment in these cases was not to ascertain the extent of coagulation, but the amount of the minimum lethal dose.

Table I.

Dose per kilogramme of body weight.	Weight of animal.	Total dose injected.	Method of injection.	Solution used.	Result.	No. of experiment.
(A) Albino rabbits injected with "albino" solution.						
	kilos.	c.c.				
16·0	0·6	10·0	In one dose	C^2	V.P.	3
12·5	0·8	10·0	„	F^7	V.P.	12
0·28	1·75	0·5	In 0·25 c.c. doses at 2′ intervals...	L^0	P. ?	77
0·6	0·5	0·3	In one dose, slowly	L^0	F.P.	78
0·27	0·9	0·25	„ „	L^0	A.B′.	80
0·31	0·8	0·25	„ „	L^0	V.F.	81
0·83	0·6	0·50	In 0·25 c.c. doses at 2′ intervals...	L^0	V.F.	82
0·45	0·55	0·25	In one dose, slowly	M^0	V.F. ?	85
0·294	1·70	0·50	In 0·25 c.c. doses at 2′ intervals...	M^0	V.F. ?	87
0·50	0·5	0·25	In one dose, slowly	M^0	F.P.	88
0·45	2·2	1·0	In 0·25 c.c. doses at 2′ intervals...	M^0	V.F.	91
0·4	0·5	0·2	In one dose, slowly	M^0	V.F.	93
0·28	1·75	0·5	In 0·25 c.c. doses at 2′ intervals...	M^0	V.F. ?	95
0·23	0·43	0·1	In one dose	M^0	V.F.	97
0·32	1·55	0·5	In 0·25 c.c. doses at 2′ intervals...	M^0	F.P.	99
0·50	0·5	0·25	In two 0·1 + one 1·5 c.c. doses at 2′ intervals	M^0	V.F.	100
0·235	1·7	0·4	In 0·2 + 0·1 + 0·1 c.c. doses at 2′ intervals	P^0	A.B.	140
0·64	0·85	0·55	In one 0·1 + three 0·15 c.c. doses at 2′ intervals	P^0	V.F.	141
0·91	0·55	0·50	In one dose	S^2	F.P. ?	164
1·1	0·45	0·50	In 0·1 c.c. doses at 2′ intervals ...	S^2	A.B. ?	165
(B) Albino rats injected with "albino" solution.						
20·0	0·5	10·0	In one dose	A^2	V.P.	2
	—	8·0	„	C^7	V.P.	5
	—	7·0	„	C^7	V.P.	7
33·0	0·12	4·0	„	F^2	P.	10

Table II.

Dose per kilogramme of body weight.	Weight of animal.	Total dose injected.	Method of injection.	Solution used.	Result.	No. of experiment.
(A) Albino rabbits injected with "pigmented" solution.						
	kilos.	c.c.				
5·0	2·0	10·0	In one dose, slowly	I^0	P.	24
4·0	2·0	8·0	" "	I^0	P.	25
2·5	2·8	7·0	" "	I^0	A.	26
6·1	1·8	11·0	" "	I^0	P.	27
1·0	1·5	1·5	" "	I^5	A.B.	29
2·0	1·3	2·6	" "	I^5	A.B.	30
2·1	1·9	4·0	" "	I^5	V.P.	31
1·25	1·6	2·0	" "	I^5	P.	33
1·15	1·3	1·5	" "	I^5	P.	35
1·0	1·7	1·7	" "	I^5	P.	37
4·66	1·2	5·6	" "	I^7	F. ?	40
0·5	2·0	1·0	" "	I^7	P.	41
7·69	1·3	10·0	" "	I^7	V.P.	42
0·52	1·9	1·0	" "	J^2	A.	53
15·7	0·7	11·0	In 0·25 + 0·5 c.c. doses at 2′ intervals	J^2	V.F.	54
5·9	1·95	11·5	In 1 c.c. doses at 2′ intervals	J^2	V.F.	56
0·41	1·2	0·5	In one dose	K^0	A.B. ?	65
1·0	1·5	1·5	In two 0·5 + one 0·25 + three 0·75 c.c. doses at 2′ intervals	K^0	V.F.	66
4·1	1·7	7·0	In 0·25 and 0·5 c.c. doses at 2′ intervals	K^0	F.	67
2·82	0·85	2·4	In 0·3 and 0·5 c.c. doses at 2′ intervals	K^2	V.F.	68
6·8	0·8	5·5	In 0·5 c.c. doses at 2′ intervals	K^2	V.F.	69
5·7	0·7	4·0	" " "	K^2	A.B. ?	70
21·4	0·7	15·0	In 1·0 and 0·5 c.c. doses at 1′ intervals	K^2	A.	71
4·28	0·7	3·0	In 2·0 + 1·0 c.c. doses at 1′ intervals	K^2	A.B.	72
12·5	0·6	7·5	In 1·5 and 1·0 c.c. doses at 1′ intervals	K^2	V.F.	73
23·6	0·55	8 of K + 5 of J	Continuously, but very slowly	K^2 & J^7	V.F.	74
10·0	0·6	6·0	" " "	J^7	V.F.	75
9·0	0·5	4·5	In one dose, quickly	J^7	V.P. nearly	76
0·31	0·8	0·25	" slowly	N^0	V.F. ?	101
0·13	1·8	0·25	" "	N^0	V.F.	103
0·38	1·7	0·65	In 0·35 + 0·3 c.c. doses at 2′ intervals	N^0	V.F. ?	105
0·5	1·0	0·5	In 0·25 c.c. doses at 2′ intervals	N^0	V.F.	107
0·41	1·2	0·5	" " "	O^0	V.F. ?	109
0·14	0·9	0·13	In one dose	O^0	V.F.	111
0·31	0·8	0·25	"	O^0	V.F.	115
0·58	0·6	0·35	In 0·2 + 0·15 c.c. doses at 2′ intervals	O^2	V.F. ?	121
0·40	0·5	0·20	In one dose	T^0	A.	173
3·80	2·85	11·0	In 0·25 c.c. doses at 1′ intervals	T^0	V.F. ?	174
(B) Albino rats injected with "pigmented" solution.						
	Not weighed	Not recorded	In one dose	B^5	A.B. ?	6
	"	4·0	"	B^5	F.P.	13
	"	5·0	"	H^0	P.	14
	"	6·0	In 4 + 2 c.c. doses	D^{12}	P.	17
	"	8·0	In small doses at 20″ intervals	D^{12}	P. or V.P.	18

Table III.—Pigmented Rabbits injected with "Albino" Solution.

Dose per kilogramme of body weight.	Weight of animal.	Total dose injected.	Method of injection.	Solution used.	Result.	No. of experiment.
	kilos.	c.c.				
21·0	2·0	42·0	In doses of 10 + 10 + 11 + 11 c.c., quickly	A^2	F.P.	1
9·09	1·1	10·0	In one dose	C^2	V.P.	4
12·5	0·4	5·0	„	F^2	V.P.	11
0·35	1·4	0·5	In 0·25 c.c. doses at 2′ intervals...........	L^0	P ?	83
0·34	1·45	0·5	„ „ „	L^0	V.F.	84
0·31	0·8	0·25	In two equal doses „	M^0	V.F.	86
0·185	1·35	0·25	In one dose	M^0	A.B′.	89
0·28	0·85	0·24	In two equal doses at 2′ interval	M^0	F.P. ?	90
0·19	1·3	0·25	In one dose	M^0	A.B′. ?	92
0·2	2·5	0·50	In 0·25 c.c. doses at 2′ intervals...........	M^0	V.F.	94
0·19	1·3	0·25	In one dose	M^0	V.F.	96
0·5	1·0	0·5	In 0·25 + 1·25 + 1·25 c.c. doses at 2′ intervals	M^0	V.F.	98
0·089	1·12	0·1	In one dose	P^0	Nearly A	136
0·083	1·2	0·1	„	P^0	Nearly A	137
0·19	1·3	0·25	In 0·1 + 0·15 c.c. doses at 2′ intervals ...	P^0	V.F.	138
0·19	1·05	0·20	In 0·1 „ „	P^0	V.F. ?	139
2·0	1·5	3·0	„ „ „	P^2	No result	145
1·3	0·75	1·0	„ „ „	P^2	V.F. ?	146
1·56	0·8	1·25	In 0·25 „ „	P^5	F. ?	149
0·57	1·3	0·75	„ „ „	P^5	V.F.	150
4·5	1·0	4·5	In 0·25 + 0·2 „ „	P^8	No result	151
0·13	1·1	0·15	In one dose	S^0	V.F.	154
1·77	2·25	4·0	In 0·2 c.c. doses at 2′ intervals	S^0	V.F. ?	155
0·7	1·0	0·7	In 0·1 „ „	S^0	V.F.	156
0·5	0·6	0·3	„ „ „	S^2	V.F. ?	161
6·3	0·95	6·0	In 0·1 c.c. and then 0·5 c.c. doses at 2′ intervals	S^2	No result	162
0·41	0·6	0·25	In one dose	S^2	Nearly A	163
11·0	1·8	20·0	In 2·0 c.c. doses at 2′ intervals	S^5	No result	169
20·0	0·55	11·0	In 1·0 „ „	S^5	No P.M. exam.	170

Table IV.—Pigmented Rabbits injected with "Pigmented" Solution.

Dose per kilogramme of body weight.	Weight of animal.	Total dose injected.	Method of injection.	Solution used.	Result.	No. of experiment.
	kilos.	c.c.				
9·0	0·5	4·5	In one dose	B^{10}	F.P. ?	16
2·8	2·1	6·0	„ slowly	I^0	V.F.	19
4·6	1·5	7·0	„ „	I^0	P. ?	20
4·0	2·5	10·0	„ „	I^0	V.F. ?	21
4·0	2·7	11·0	„ „	I^0	P.	22
3·0	2·0	6·0	„ „	I^0	F.P.	23
1·07	1·4	1·5	„ „	I^5	F.P.	32
1·5	2·0	3·0	„ „	I^5	A.B′.	34
0·97	1·85	1·8	„ „	I^5	F.P. ?	36
0·76	1·3	1·0	„ „	I^7	P.	43
3·8	1·3	5·0	„ „	I^{19}	F.P.	46

Table IV—*continued.*

Dose per kilogramme of body weight.	Weight of animal.	Total dose injected.	Method of injection.	Solution used.	Result.	No of experiment.
	kilos.	c.c.				
4·6	1·5	7·0	In one dose, slowly	I^{21}	V.P.	47
0·52	1·9	1·0	,, ,,	J^2	V.F.	57
4·28	2·1	9·0	In 1 c.c. doses at 2′ intervals	J^5	V.F. ?	58
0·5	1·0	0·5	In one dose, slowly	K^0	P.	59
0·55	0·9	0·5	,, ,,	K^0	V.P.	60
0·5	1·0	0·5	,, ,,	K^0	F.P. ?	61
0·37	0·8	0·3	,, ,,	K^0	V.F.	62
3·1	0·8	2·5	In 0·25 + 0·5 c.c. doses at 2′ intervals ...	K^0	V.F.	63
0·5	1·0	0·5	In one dose, slowly	K^0	P.	64
0·12	2·05	0·25	,, ,,	N^0	V.F.	102
0·25	0·8	0·2	In 0·1 c.c. doses at 2′ intervals	N^0	V.F. ?	104
0·25	1·0	0·25	In 0·1 + 1·5 c.c. doses at 2′ intervals	N^0	V.F. ?	106
0·26	1·9	0·5	In 0·25 c.c. doses at 3′ intervals	O^0	V.F. ?	108
0·30	1·2	0·37	In 0·25 + 0·12 c.c. doses at 2′ intervals ...	O^0	V.F.	110
0·20	1·0	0·20	In 0·1 c.c. doses at 2′ intervals	O^0	V.F.	113
0·12	0·8	0·1	,, ,, ,,	O^0	V.F.	114
0·45	0·55	0·25	In 0·1 + 0·15 c.c. doses at 2′ intervals ...	O^2	V.F. ?	116
0·24	1·25	0·30	In 0·15 c.c. doses at 2′ intervals	O^2	V.F. ?	117
0·41	0·6	0·25	In 0·1 + 0·15 c.c. doses at 2′ intervals ...	O^2	V.F. ?	118
0·38	0·65	0·25	,, ,, ,, ...	O^2	V.F. ?	119
0·5	0·6	0·3	In 0·1 c.c. doses at 2′ intervals	O^2	V.F. ?	120
0·31	0·8	0·25	In 0·1 + 0·15 c.c. doses at 2′ intervals ...	O^2	V.F.	122
0·41	0·6	0·25	,, ,, ,, ...	O^5	V.F.	128
0·27	0·9	0·25	,, ,, ,, ...	O^5	V.F.	129
0·173	1·15	0·2	In 0·1 + 0·1 ,, ,, ...	O^5	V.F.	130
0·158	0·63	0·1	In one dose	O^5	V.F.	131
0·25	2·0	0·5	In 0·1 c.c. doses at 2′ intervals	O^5	V.F.	132
0·28	1·25	0·35	In two equal doses at 2′ intervals	O^7	F.P.	133
0·178	1·4	0·25	,, ,, ,,	O^7	F.P.	134
0·25	0·6	0·15	In one dose	O^7	V.F.	135
0·8	0·5	0·4	In three equal doses at 2′ intervals	O^9	V.F.	142
1·0	0·6	0·6	In five ,, ,, ,,	O^9	F.P. ?	143
0·91	0·55	0·5	In three ,, ,, ,,	O^9	V.F. ?	144
1·22	1·43	1·75	In 0·25 c.c. doses at 2′ intervals	O^{11}	V.F.	147
7·5	1·0	7·5	,, ,, ,,	O^{11}	V.F.	148
0·37	1·35	0·5	In 0·1 ,, ,,	R^0	V.F.	152
0·26	0·95	0·25	In 0·1 + 0·15 ,, ,,	R^0	V.F.	153
16·8	1·48	25·0	In 0·1 + 0·25 + 0·5 + 1·0 c.c. doses at 2′ intervals	R^2	No result	157
0·71	0·7	0·5	In 0·25 c.c. doses at 2′ intervals	R^2	F.P. ?	158
0·73	1·7	1·25	,, ,, ,,	R^2	V.F.	159
1·3	0·75	1·0	In 0·1 ,, ,,	R^2	V.F.	160
1·29	0·85	1·1	,, ,, ,,	R^5	No P.M. exam.	166
2·9	1·7	5·0	In 0·25 ,, ,,	R^5	,,	167
13·3	0·6	8·0	In 0·1 + 0·25 ,, ,,	R^5	,,	168
0·41	1·95	0·8	In 0·1 ,, ,,	T^0	P ?	171
3·1	0·86	2·75	,, ,, ,,	T^0	V.F. ?	172
1·18	1·9	2·25	In 0·25 ,, ,,	T^2	P ?	175
1·6	1·4	2·25	,, ,, ,,	T^2	V.F.	176
2·32	1·4	3·25	,, ,, ,,	T^5	No P.M. exam.	177
1·13	1·1	1·25	,, ,, ,,	T^5	,,	178
2·6	1·05	2·75	,, ,, ,,	T^5	,,	179
3·0	0·75	2·25	,, ,, ,,	T^5	V.F.	180

Table V.

Dose per kilogramme of body weight.	Weight of animal.	Total dose injected.	Method of injection.	Solution used.	Result.	No. of experiment.
(A) Himalayan rabbits injected with "pigmented" solution.						
	kilos.	c.c.				
0·43	2·3	1·0	In one dose	I^5	A.	28
4·34	2·3	10·0	"	B^8	A.B	8
4·10	1·7	7·0	In 0·7 c.c. and in 0·5 c.c. at 2′ intervals	J^2	V.F.?	49
0·52	1·9	1·0	In one dose	J^2	F.	50
1·57	1·9	3·0	In one 1 c.c. and in 0·5 c.c. at 1′ intervals	J^2	V.F.	51
1·87	1·6	3·0	In one 0·8 c.c. and in 0·2 c.c. at 2′ intervals	J^2	V.F.	52
0·18	2·7	0·5	In 0·25 c.c. at 2′ intervals	O^2	V F.?	112
0·62	0·4	0·25	In two 1·5 + 1 c.c. at 2′ intervals	O^2	V.F.?	123
(B) Himalayan rabbits injected with "albino" solution.						
4·76	2·1	10·0	In one dose	G^2	V.P.	9
0·5	0·5	0·25	"	L^0	P.	79

REFERENCES.

1889. Altmann. 'Arch. f. Physiol.,' Leipzig, 1889.

1899. Darwin, Charles. 'Variation of Animals and Plants under Domestication 2nd edit., vols. 1 and 2, London, 1899.

1903. Farabee and Castle. 'Science,' N.S., vol. 17, New York.

1880. Foà and Pellacani. 'Riv. clin. di Bologna,' 1880.

1893. 1895. 1896. 1897. Halliburton, W. D., and Brodie, T. G. 'Journ. Phys.,' Cambridge and London, vols. 14, 16, 17, and 18.

1897. Halliburton and Pickering. 'Journ. Phys.,' vol. 18.

1846. Heusinger. 'Wochenschrift fur die Heilkunde,' Berlin, 1846.

1894. Martin, C. 'Journ. Phys.,' Cambridge and London, vol. 15.

1874. Miescher. 'Verhandl. d. naturf. Gesellsch. in Basel,' 1874, Part 1.

1896. Miescher and Schmiedeberg. 'Arch. f. exper. Path. u. Pharmakol,' Leipzig, 1896 vol. 37.

1907. Mudge, George P. 'Encyl. Brit.,' art. "Albino," 10th edit.

1897. 1899. Pickering, 'Journ. Phys.,' vols. 18 and 20.

1855. Prichard, Jas. C. 'Natural History of Man,' London, 1855.

1897. Ringer. 'Journ. Phys.,' Cambridge and London, vol. 18.

1898. Schäfer, E. A. 'Text Book of Physiology,' London, 1st edit., vol. 1.

1903. Stephens and Myers, 'Journ. Phys.,' vol. 23.

1845. Twining. 'Brit. Assoc. Reports,' 1845.

1886. Wooldridge. 'Proc. Roy. Soc. London,' 1886.

1888. Wooldridge. 'Arch. f. Physiol.,' Leipzig, 1888.

1891. Wright. 'Journ. Phys.,' vol. 12.

The Chemistry of Globulin.

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The object of the present paper is first to establish simple formulæ for the more important of the experimental results obtained by Hardy* and Mellanby,† then to interpret these in their bearing upon the chemistry of globulin in connection with a theory of colloids, and finally to find the molecular mass (weight) of globulin. The work will be taken according to the following table of contents.

1. Formula for the solution of globulin in solutions of neutral salts, and the laws associated with this formula.
2. Solution of globulin in acids and alkalies.
3. Formulæ for the precipitation of globulin by salts and by acids.
4. A theory of the colloidal state.
5. The electric conductivity of globulin solutions.
6. The molecular mass (weight) of globulin.

1. *Formula for the Solution of Globulin in Solutions of Neutral Salts, and the Laws Associated with this Formula.*

Mellanby sets forth his results in graphs, from which he makes some broad deductions, to be discussed later in this section. His most striking result, obtained also by Hardy, is that the fraction (or percentage) of a globulin suspension which is dissolved by a salt solution of given strength is nearly independent of the original strength of the suspension. In other words, the amount of globulin dissolved by a given salt solution is proportional to the amount of globulin presented for solution in the form of a suspension. This fact is of fundamental importance for the colloidal globulin of the suspension. For example, a 0·3-per-cent. NaCl solution dissolves from a 3·04-per-cent. suspension of globulin in 100 c.c. 1·12 grammes, from 1·68 per cent. 0·64, from 1·06 per cent. 0·39, and from 0·71 per cent. 0·30.

Let G be the original strength of a globulin suspension when it is instantaneously distributed through a solution of a salt of concentration c, G and c being given in grammes per cubic centimetre. Let g be the grammes of globulin per cubic centimetre dissolved from the suspension when saturation is reached, and let C be the concentration of the salt required to just dissolve the whole of G. Denote g/G by p, and c/C by q, then Mellanby's results are expressed by the equation

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* 'Journal of Physiology,' 1905, vol. 33, p. 251.

† *Loc. cit.*, p. 338.

$$p(1-q) = Aq(1-p), \tag{1}$$

in which A is a parameter having characteristic values for each type of neutral salt. To illustrate the applicability of this equation, the following comparison is made of the values of $p(1-q)/q(1-p)$ for the different values of c and p given by Mellanby for his first set of data for NaCl. C was not actually measured by Mellanby, but is obtainable from his first graph with only a small extrapolation as 0·0052. Hence, from Mellanby's tabulated values of c, we obtain the values of q, given in the table along with his values of p. The last row contains the ratio $p(1-q)/q(1-p)$.

Table I.

10^4c	50	45	40	35	30	25
10^3q	962	865	769	673	577	481
10^3p	910	830	665	555	450	356
$10^2\dfrac{p(1-q)}{q(1-p)}$	40	76	60	61	60	60

The first two values are aberrant, because $1-q$ and $1-p$ being small are powerfully affected by experimental uncertainty. If we take $p(1-q)/q(1-p) = 0{\cdot}60 = A$, and use the equation to calculate p from q, we find for p in these first two cases 0·938 and 0·794 in place of 0·91 and 0·83, the differences being within the limits of experimental error.

Mellanby shows that the graphs for NaCl, KCl, and NH_4Cl coincide when c expressed as gramme equivalents is made abscissa and p ordinate. Hence the value of A just found for NaCl, namely 0·60 holds for KCl and NH_4Cl. Mellanby generalises this result for all neutral salts composed of two monovalent ions, but in the appendix to his paper he points out that the graphs for $K\bar{A}$, KBr, and KI do not coincide with that for KCl, a result previously found by T. B. Osborne, and given also by Hardy. Mellanby finds that a single graph will suffice for Na_2SO_4, $K_2SO_4(NH_4)_2SO_4$, $MgCl_2$, $BaCl_2$, and $CaCl_2$. For this type, namely, a divalent and two monovalent ions, I find $A = 0{\cdot}613$, say 0·61, and for the type $MgSO_4$ $A = 0{\cdot}66$.

One of Mellanby's discoveries leads towards a useful generalisation in connection with A. He measures the average solvent power of any salt for globulin up to the point when the whole of a suspension is dissolved, and finds this proportional to the sum of the squares of the valencies of its ions. For example, if the solvent power of Na is 1^2 and of Cl is 1^2, that of NaCl will be 2, that of $MgCl_2$ will be 2^2+2 or 6, and that of $MgSO_4$ 2^2+2^2 or 8. It seems simpler to measure solvent power by the reciprocal of the concentration required just to dissolve the whole of a given globulin suspension. From Mellanby's graphs we find this concentration C to be 0·000094 E for salts of the type NaCl, E being the gramme equivalent. For the type $CaCl_2$ and Na_2SO_4 it is 0·000065 E and for $MgSO_4$ 0·000056 E. Hence, for 1/C for these three types, we get the values 10600/E, 15400/E, and 17850/E. If we use gramme molecular mass M instead of gramme equivalent E, these become 10600/M, 30800/M, and 35700/M, in which the numbers are as 2 : 6 : 7, corresponding to the ratios 2 : 6 : 8 found by Mellanby when using average solvent powers. We can regard this result in a different way. Let us write (1) in the form

$$p\left(1-\frac{c}{C}\right)=\frac{A}{C}c\,(1-p), \qquad (2)$$

and study A/C for each type, thus making the absolute concentration c prominent. The values of A/C for the three types are 6400/M, 18800/M, and 23600/M, the numerical parts of which are MA/C, and are more nearly as 2 : 6 : 8. From this point of view, we find Mellanby's law apply better to MA/C than to M/C, to an average value of which Mellanby applied it. In Section 4, I shall try to give a theoretical explanation of Mellanby's law applied to MA/C. Meanwhile, to complete the discussion of experiments bearing on A, we shall consider his results concerning the influence of temperature on the solvent power of NaCl for globulin. He comes to the conclusion that the solvent power is proportional to the electric conductivity or summed ionic velocities of the solution of NaCl at the temperatures used. But it will be better to investigate the effect of temperature on A and A/C, and then follow Mellanby in taking account of ionic velocities in this connection.

The following table contains the values of A which I have calculated from Mellanby's data* at the five temperatures, also the values of C/E derived from his graphs. The values of EA/C are also tabulated to give relative values of A/C. The column marked η gives the viscosity of water at the five temperatures, and the last column ηEA/C :—

Table II.

Temp.	10^3A.	10^6C/E.	EA/C.	$10^4\eta$.	ηEA/C.
° C.					
1·5	860	168	5120	172	88
20	600	102	5860	101	59
30	536	84	6310	80	50
40	512	75	6790	66	45
50	605	71·5	8470	55	47

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* *Loc. cit.*, p. 353.

The value of A attains a minimum between 30° and 50°, probably between 30° and 40°. It is worth noticing that this minimum comes near to the temperature of warm-blooded animals. The viscosity of water controls the velocities of an ion at different temperatures and is inversely proportional to them. So the column headed $\eta EA/C$ gives relative values of the ratio of EA/C to the velocities of Na and Cl at the five temperatures.

These show a minimum about 40° and can be expressed by

$$\eta EA/C = 45 + 0{\cdot}031\,(t-40)^2. \tag{3}$$

This equation makes the value of A/C at different temperatures depend not only on the viscosity η, which determines the velocities of the ions, but on specific properties of the globulin, which are a function of the temperature. Certainly one would expect, *a priori*, that the profound dependence of the chemical and physical properties of proteid on temperature would appear in such an equation; one would expect the approaching coagulation at 75° to be foreshadowed. It is again striking that the temperature of minimum $\eta EA/C$ is about 40°, near that of warm-blooded animals. These experiments have yielded in equation (1) the result that the chemical equilibrium between globulin and neutral salts is of a simple type, dependent, as far as the salt is concerned, upon ionic electric charges and velocities, but also dependent upon special properties of globulin.

It has already been mentioned that $K\bar{A}$, KBr, and KI are exceptional to a certain extent and show that other properties of salts than ionic charges and velocities have an influence on their chemical equilibrium with globulin. Moreover, the solvent powers found by Hardy* for globulin from ox blood do not agree closely with those given by Mellanby for horse globulin. For instance, Hardy finds $MgSO_4$ less powerful than K_2SO_4, whereas Mellanby finds its power 8/6 of that of K_2SO_4. Hardy finds KNO_3, $NaNO_3$, and NH_4NO_3 1·5 times as powerful as KCl and NaCl. Yet Hardy's data on the whole show that valency is the chief factor controlling solvent power. He finds K and NH_4 oxalates to have the same power as $(NH_4)_2SO_4$ and an equivalent of Na citrate 3·3 times as powerful as NaCl, so the gramme molecule would be 9·9 times as powerful as that of NaCl. On Mellanby's principle it would be $(3^2+3)/2$ or six times as powerful. Further investigation must clear up these points.

2. *Solution of Globulin in Acids and Alkalies.*

Hardy's experiments show that the reactions between globulin on the one hand and acids and alkalies on the other take place under the usual chemical conditions of combination. Whereas the amount of a globulin suspension dissolved by a certain strength of NaCl is proportional to the original concentration of the suspension, the amount dissolved by HCl depends only on the total amount of acid which has access to the suspension. Hence, for acids and alkalies in place of (1), we have the relation

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* *Loc. cit.*, pp. 308 and 309.

$$g = \mathrm{B}c, \tag{4}$$

where B is a parameter characteristic of each acid and alkali. Mellanby established the same result and shows that B does not vary with temperature. The contrast between solution in neutral salts and in acids or alkalies is complete. Hardy proves that a given amount of globulin reacts not with equivalents, but with molecules of such acids as HCl, H_2SO_4, and H_3PO_4. Similarly he finds that the molecule $Ba(OH)_2$ has the same solvent power as KOH, NaOH, and NH_4OH. To dissolve one gramme of globulin 10×10^{-5} gramme equivalent of alkali is required, and 18×10^{-5} gramme equivalent of the strong acids. These results will be used in Sections 4 and 6. T. B. Osborne, from 1899 to 1901, obtained similar results for the vegetable globulin edestin.

3. *Formulæ for the Precipitation of Globulin by Salts and by Acids.*

The first case is that in which globulin is precipitated by excess of a solvent neutral salt. Let c be the concentration of the salt and p the fraction which the precipitated globulin is of the total originally in solution, then Mellanby's graph for $(NH_4)_2SO_4$* is given by the equation

$$p(1+p) = 28{\cdot}8\,(c-0{\cdot}152). \tag{5}$$

The values of c given by this for the experimental values of p are compared with the experimental values of c in the following table:—

Table III.

10^3p	315	447	533	695	787	858	1000
10^3c exp.	166	175	183	192	200	208	218
10^3c calc..........	166	174	180	193	202	207	221

To interpret this equation, we start with the consideration of the value c_0 of c at which precipitation just begins. Two actions of the salt just balance here, the solvent action and the precipitant. If, then, by increasing the concentration to c, the fraction p of the globulin is precipitated, an amount of solvent power proportional to c_0p is liberated, so the solvent power, which at c_0 was proportional to c_0, at c is proportional to $(1+p)\,c_0$. This implies that the whole of the additional salt $c-c_0$ is occupied in keeping the precipitate down. This solvent power proportional to $(1+p)\,c_0$ acting on the precipitate p will give a rate of solution of the precipitate proportional to $p\,(1+p)\,c_0$, and this must be equal to the rate of precipitation when equilibrium is reached. But as $c-c_0$ is occupied in keeping the precipitate down, the simplest assumption to make is that the rate of precipitation is proportional to $c-c_0$. Hence, for equilibrium,

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* *Loc. cit.*, p. 357.

$$p\,(1+p) = \mathrm{F}\,(c-c_0), \tag{6}$$

where F is a parameter characteristic of each salt.

This equation of equilibrium is of the same form as (5). The precipitate in this case is probably not globulin, but a compound of globulin and the salt. It begins to come down at a concentration about 40 times that which produces complete solution of the original globulin. But Mellanby's data for $MgSO_4$ cannot be expressed by an equation like (5). They could be represented by portions of two straight lines.

When a salt of a heavy metal is added to a solution of globulin in a neutral salt, the strength of the neutral salt being kept constant in all the comparisons, the fraction of the total globulin precipitated is proportional to the amount of the salt of heavy metal up to a certain point.

Thus, with globulin dissolved in 0·6 per cent. NaCl and then precipitated with $ZnSO_4$, also dissolved in 0·6 per cent. NaCl, so that the total amount of globulin in 10 c.c. was always the same, the fraction p of the globulin precipitated is given by

$$p = 2730c, \tag{7}$$

up to $c = 0{\cdot}000125$ gramme per cubic centimetre, c being here the grammes of $ZnSO_4$ per cubic centimetre of the 10 c.c. used. From $c = 0{\cdot}000125$ to $c = 0{\cdot}000225$ the formula is

$$p-0{\cdot}35 = 1030\,(c-0{\cdot}000125). \tag{8}$$

Mellanby converts these two lines in his graph into a single curve, but with some violence to the experimental data. For a solution of globulin containing 0·0027 gramme NaCl per cubic centimetre and $ZnSO_4$ as precipitant, $p = 236\,c$ up to $c = 0{\cdot}001$, while from $c = 0{\cdot}001$ to $c = 0{\cdot}0035$ we can write $p - 0{\cdot}15 = 82\,(c - 0{\cdot}001)$.

For $CuSO_4$ the corresponding equations are $p = 3660\,c$ up to $c = 0{\cdot}00015$, and $p - 0{\cdot}55 = 2000\,(c - 0{\cdot}00015)$ up to $c = 0{\cdot}00025$.

In this connection it is interesting to review the considerable amount of analytical work done on the albuminates of the heavy metals.* Harnack found, in 1881, that copper albuminate precipitated from excess of albumin contains 1·35 per cent. of Cu, and by excess of Cu 2·64 per cent. In 1877, Mörner prepared a copper albuminate with 1·2 per cent. of Cu, and another with 1·85 per cent. In 1887, Chittenden and Whitehouse obtained copper albuminate containing from 0·71 to 1·21 per cent. of Cu, but found that by redissolving and reprecipitating the percentage could be raised to 2·19. Now with our formula for $CuSO_4$, and the fact that Mellanby's globulin solution contained 0·024 gramme of globulin per cubic centimetre, we get $0{\cdot}024\ p = 88\,c$, that is to say, that at the earlier stages of precipitation $CuSO_4$ throws down 88 times its own weight of globulin. Thus, neglecting the small amount of Cu left in solution, we can calculate that the copper globulinate of Mellanby's experiment contained 0·45 per cent. of Cu. If all the globulin is precipitated, our equations give that 0·55 of the precipitate contains 0·45 per cent. of Cu, and 0·45 of it contains 0·83 per cent. of Cu, so that the precipitate as a whole contains 0·62 per cent.

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* See F. N. Schulz, 'Die Grösse des Eiweissmoleküls,' Jena, 1903.

These are only about one-third of Mörner and Harnack's values for the albuminates. But the larger value, 0·62 per cent., is close to the smallest of Chittenden and Whitehouse's, namely, 0·71. In the same way, from our equations for Mellanby's $ZnSO_4$ data, we find that the first precipitate of zinc globulinate contains 0·61 per cent. of Zn, and that if all the globulin is thrown down the compounds average 1·22 per cent. of Zn. Now Chittenden and Whitehouse found 0·91 per cent. of Zn in their zinc albuminate. So there is a fair correspondence between the composition of Mellanby's globulinates and Chittenden and Whitehouse's albuminates of Cu and Zn. Thus it bears upon the present inquiry to look more closely into the albuminates of these investigators. The following table contains, in the first row, the fraction *f* of metal in unit mass of its albuminate, in the second the equivalent mass E of the metal, and in the third the ratio *f*/E.

Table IV.

	Cu.	Zn.	Fe.	U.	Pb.	Hg.	Ag.
$10^2 f$	71	91	95	460	250	289	409
E	32	32·5	28	120	104	100	108
$10^3 f/E$	22	28	34	38	24	29	38

From the mean value of the last row we find the percentage of heavy metal in its albuminate to be 0·03 E. Hence the minimum molecular mass for albumin in the albuminates is 100/0·03 or 3300 nearly. Hence, we infer from the above argument that Mellanby's data would agree with the altogether independent data for the albuminates in making the minimum possible molecular mass for globulin about 3000. This result will be used in Section 6.

We shall now discuss the precipitation by acids of globulin dissolved in neutral salts. This is a very characteristic point in the chemistry of globulin. While a little acid dissolves globulin, it precipitates it from solution in salts. One would expect *a priori* from the similarity of salts and acids that they would help one another in dissolving globulin. Instead of that they are antagonistic. Hardy has shown that the solvent power of two neutral salts for globulin is the sum of their separate powers. All the more striking, therefore, is the antagonism of neutral salts and acids. It seems to me that the explanation of this fact is to be found in this, that the acids enter into the globulin molecule in different places from the neutral salts, because the H atom can play a different *rôle* in organic compounds from that possible to metallic atoms. The ordinary organic compounds are far more stable than the organo-metallic.

Moreover, the molecule of acid and of neutral salt enter the globulin not only in different places, but in places which make them incompatible with one another, unless special provision is made to keep them in position. Each is stable in its own place. Together they tend to instability. Hence, acids and neutral salts inhibit one another's action in dissolving globulin. We can apply this idea quantitatively to Mellanby's experiments in which acid is made first to precipitate globulin from solution in salts, and then to redissolve the precipitate. He used his solutions in such a way that the total amount of globulin and of neutral salt in the 10 c.c. of mixture under observation remained constant in a set of experiments. The strength of the neutral salts was 0·005, or 0·003 gramme per cubic centimetre, and of the globulin sufficient to saturate the salts. The amount of acid present in any case in the 10 c.c. is specified by the number of cubic centimetres of N/100 acid from which it could be obtained. The results show that up to maximal precipitation the fraction of the globulin precipitated is practically proportional to the amount of acid present. In fact, Mellanby's graphs, in which p the fraction of globulin precipitated is ordinate and amount of acid is abscissa, could be replaced by two straight lines, one running up from the origin to the point of maximum precipitation, and the other running down again to a point of zero precipitation or complete solution.

To discuss the theory of the whole action, let us denote by a the cubic centimetres of acid which in the absence of salt would just dissolve all the globulin. Let b be the number producing maximal precipitation in a given salt solution containing the globulin. Then b would dissolve the fraction b/a of the globulin in the absence of salt. But the salt present is able just to dissolve all the globulin by itself, hence we may say that of the total solvent power of the salt and the acid b, the salt and acid possess the fractions $1/(1+b/a)$ and $(b/a)/(1+b/a)$ respectively. So if p_m is the maximum fraction of the globulin precipitated by b, then $1-p_m$ is the fraction held in solution by acid, and the fraction held in solution by the salt is $\frac{1-p_m}{1+b/a}$.

Hence, by the presence of the acid b the salt is deprived of $1-(1-p_m)/(1+b/a)$ of its solvent power, and this gives us a measure of the inhibition. The amount of acid required to inhibit all the salt will be $b\div\{1-(1-p_m)/(1+b/a)\}$. But to dissolve all the globulin as well as to inhibit the salt we shall need an additional a of acid. Let r be the total experimental amount of acid that will just give a clear solution again, then

$$\frac{b}{1-(1-p_m)/(1+b/a)}+a=r. \qquad (9)$$

This is an equation for calculating a from the observed values of p_m, b, and r. For a solution containing 0·005 gramme of NaCl per cubic centimetre, with HCl and H_2SO_4 as acids, Mellanby found $p_m = 0{\cdot}15$ and $b = 2{\cdot}5$, while $r = 7{\cdot}3$. Solving (9) for the positive root of a, we get $a = 2{\cdot}8$. From all his data the following table has been prepared for four salt solutions of the strength specified:—

Table V.

	NaCl 0·5 per cent.	Na_2SO_4 0·5 per cent.	$MgSO_4$ 0·3 per cent.	$MgCl_2$ 0·3 per cent.
10^2p_m	15	9	17	7
$10b$	25	45	40	39
$10r$	73	90	100	67
$10a$	28	25	39	15

These strengths of solutions dissolve nearly equal amounts of globulin in 10 c.c., but not quite equal. If we calculate the relative amounts of globulin by the principles of Section 1, we find that they are as 1·7, 2·1, 2·0, and 1·9. Hence, relative values of a, referred to equal amounts of globulin, will be derived from the values of $10a$ in the table by dividing by these numbers, giving 17, 11·4, 17·5, and 9. Now these are to one another nearly as the values of 10^2p_m in the table, a fact which confirms in a general way the above reasoning and calculation, though, strictly, instead of comparing with p_m, we should compare with p_m referred to equal mass of globulin for each salt. In a broad way a tends to be proportional to p_m.

It is a striking fact that though the acids HCl and H_2SO_4 act by equivalents in each case, the same number of equivalents is not required in different cases; in other words, a for different solvents and a constant amount of globulin is not a constant, although with pure globulin these acids react in definite proportions. It seems, indeed, as though the precipitate we are now discussing cannot be pure globulin. So it is noteworthy that the amounts of acid required to yield p_m are nearly proportional to the cube of the total globulin strength in each of the above four cases, for, taking the values of $10b$ from Table V and dividing them by the cubes of the relative globulin strengths given above, we get $25/1{\cdot}7^3$, $45/2{\cdot}1^3$, $40/2^3$, and $35/1{\cdot}9^3$, or 5·1, 4·9, 5·0, and 5·1. It would appear, then, that the equation of chemical equilibrium between acid b and total globulin G, dissolved in neutral salt, is of the form

$$G^3 = kb. \tag{10}$$

Here, evidently, G^3 represents the mass action amongst three globulin compounds, whose amount in each case at the point of maximal precipitation is proportional to the total amount of globulin present. Of these three we know that two are probably the compound of globulin with acid alone and the compound with neutral salt alone. The third, then, is probably a compound containing both acid and salt, stable only when the other two are present in excess. These compounds form by their joint action the precipitate, whose rate of formation is measured by G^3. Then in (10) kb must represent the rate of action of the acid tending to dissolve the precipitate, which acts in the usual way with a constant active mass.

It is worth noting that when NaCl and $MgSO_4$ are the solvents, acids precipitate globulin nearly equally, while when Na_2SO_4 and $MgCl_2$ are the solvents, acids precipitate nearly equal fractions, but smaller than in the previous case. It seems as if ions of the same valency, like Na and Cl or Mg and SO_4, can co-operate better than dyad with monad as found in Na_2SO_4 and $MgCl_2$. This recalls the observation of Picton and Linder, that ions of different valency inhibit one another in precipitating colloids, while ions of the same valency co-operate.

4. *A Theory of the Colloidal State.*

The characteristic of the colloid state is that the molecules cease to have a separate existence; they link on to one another by means of the atomic electric charges, thus forming the meshes so characteristic of colloids

Each particle in a suspension of a globulin might, therefore, be called a molecule, but with no advantage. In each such particle, however, a certain pattern is repeated in three-dimensional space. In a colloid this pattern is the real subject of the chemist's study, just as the molecule is in crystalloids. Because of its importance, I propose to call this pattern a semplar. An imaginary case will enable the theory to be presented in a definite form. The nitrogen of NH_3 acts trivalently, but it can act also with pentad valency. In the old phraseology two of the bonds of N in NH_3 were said to satisfy one another. Now, in Helmholtz's theory, valency is the number of electrons associated with an atom. I have proposed to consider variable valency as due to the existence of opposite electrons in the atom showing it,* and for convenience of notation and printing I have proposed to denote the positive electron by ♯ and the negative by ♭. Thus both the triad and pentad valencies of N are expressed by writing it $\sharp N \flat_4$, because ♯ and ♭ can coalesce to form a doublet ♯♭, in which each electron almost completely neutralises the chemical activity of the other. On the same principle the symbol of the hydrogen ion is H♯, so that in NH_3 there are three doublets ♯♭ chemically effective and one ineffective. I have shown that these doublets and others are the cause of cohesion and rigidity. In a crystal of frozen NH_3 the molecules are all separate, each affecting its neighbours by the action of these doublets upon one another. But imagine each ineffective doublet of N to be made effective, so that ♯ reaches out to unite with ♭ of a neighbour N, then every molecule of NH_3 becomes chemically linked with two of its neighbours, it becomes a semplar, and the whole crystal becomes a mesh of such semplars. Under certain conditions it could retain its crystalline form. This imaginary example explains the nature of a colloid on the present theory, and shows how the transition from crystalloid to colloid can be continuous with occurrence of crystalline colloids. The formation of polymers is due to ineffective doublets becoming effective, though generally the linking up of molecules is restricted to groups of m molecules, where m measures the amount of polymerisation. The most important case is that of water, which I have shown† to be a mixture of $(H_2O)_3$ trihydrol, found pure in ice, and of $(H_2O)_2$ dihydrol. In water and other polymers we have the simplest instances of semplars. When m, the measure of polymerisation, becomes large and indefinite, it gives us the colloid state, in which chemical forces between neighbour semplars produce effects of the same order as those between the separate doublets of molecules in the solid state. Before

* 'Phil. Mag.,' [6], vol. 3, p. 161.

† 'Phil. Mag.,' [5], vol. 50, p. 460.

applying this theory to the chemistry of globulin, it will be helpful to discuss with its aid the remarkable connection, discovered by Schulze and investigated by Picton and Linder, between the valency of ions and their coagulating power. Hardy has shown that a similar relation holds for certain organic colloids. A theoretical explanation of it has been offered by Whetham,* based upon considerations as to the probability of electrolytic ions of like sign coming together. Though suggestive, this theory seems to me inadequate, because the electric repulsion between ions of like sign tends powerfully to keep them apart. On the present theory, coagulation consists in opening out ineffective doublets ♯♭ so that they link together neighbour molecules of the coagulating substance. Hence ionic charges must sometimes be instrumental in coagulation, because a free ♯ tending to attract ♭ in the doublet and to repel ♭ is an agent for opening out ineffective doublets. Suppose that a free ♯ unites with ♭ from an ineffective ♯♭ of a molecule of coagulee, then it liberates ♯, which will grasp the ♭ of a neighbouring ineffective ♯♭, and so the coagulation spreads until perhaps the other ion of the electrolyte with its electron ♭ steps in and arrests the process. But there is another way in which coagulation can be propagated from an ion as centre. The electric force of the ion will act on the ineffective doublets out to a considerable distance from itself, and, moreover, every ineffective doublet which is made chemically effective alters the local electric equilibrium by the change. Each coagulated molecule is a centre of coagulation. Hence we have the speed of coagulation due to an ion proportional to the electric charge of the ion and to the amount of coagulation already produced by it. Let c be the coagulum produced by electrolyte of concentration s, then we have, with ν for valence of the effective ion and k a constant,

$$\frac{d}{dt}\left(\frac{c}{s}\right) = k\nu\frac{c}{s} \quad \therefore \quad \log c/c_0 = k\nu\,(t-t_0), \tag{11}$$

where c_0 is the amount of coagulum produced at a certain short time t_0 after the beginning of the process. This small initial coagulation is produced mostly by the first process of direct linking, and is proportional to νs the total number of electrons which start the linking up, so we may write

$$\log c/\nu s = k\nu\,(t-t'_0). \tag{12}$$

Now, in the experiments, the number of equivalents νs of electrolyte required to produce a certain amount of coagulation c in a very short time are measured. We may neglect t'_0 and take t as well as c to be the same for each electrolyte. If we call $1/\nu s$ the coagulating power of the electrolyte, equation (12) makes its logarithm linear in the valency ν. Schulze found the coagulative powers of chlorides of mono-, di-, and tri-valent ions on arsenious sulphide to be as 1 : 30 : 1650, the common logs of which are 0, 1·477 and 3·217, which fall nearly into the linear form 1·564 $(\nu-1)$.

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* 'Phil. Mag.,' [5], vol. 48.

The data of Linder and Picton for the corresponding sulphates on arsenious sulphide are as 1 : 35 : 1023, whose logs are 0, 1·544, and 3·01, which may be written 1·515 $(\nu-1)$.

The present theory, then, lends itself well to an explanation of this remarkable law.

In the chemistry of globulin, the prominent fact is that salt solutions dissolve from a suspension an amount nearly proportional to the original total strength of the suspension. According to the present theory, the explanation is as follows: Just as ions by their elective force draw out the ineffective doublets of molecules, which they cause to join up as semplars, they can, under suitable energy conditions, break the doublets joining the semplars, which they liberate as molecules. For example, if a crystal of our imaginary colloid NH_3 were dissolved in HCl, the action would be one in which the ions H and Cl would break the doublets joining the semplars NH_3, and would join on to form NH_4Cl. This I take to be a representation of the solution of globulin in acids, the action of salts being not so simple. It is a corollary of the coagulation of colloids by electrolytes. The same electric actions that build up a coagulum can sometimes pull it down, especially if the operating ions can step into places which make the total chemical potential energy smaller than before. Now the number of semplar doublets in a globulin suspension which the ions can break will either be the total thereof or a certain fraction of the total. In the former case we should have solution of the colloid in the ordinary way, as by acids. In the latter case the ions will only break those doublets which can be made unstable by the particular attacking force of ions used. Hence the fraction of a globulin particle dissolved by a given solution will have a definite value, and the amount of globulin dissolved will be proportional to the total number of particles, that is, to the original concentration of the suspension. This is the explanation of the most striking experimental result brought out by Hardy and Mellanby. We now proceed to give the corresponding interpretation of equation (1) deduced from Mellanby's experiments. Let us denote the semplar of globulin by G. The simplest possible formula for the semplar at the moment of its detachment is $\flat G\sharp$. Then either $\sharp$ and $\flat$ can coalesce to form an ineffective doublet, leaving G as a molecule, or if ions like Na and Cl are present, their electric charges will link them on, giving $Na\sharp\flat G\sharp\flat Cl$, or, more briefly, NaGCl. Now the fact that about 150 times as many equivalents of NaCl as of HCl are required to keep a given amount of globulin in solution, shows that NaGCl must be relatively very unstable; a large excess of Na and Cl ions is required to keep it in existence. Its existence depends on the breaking of doublets and keeping them broken. The number of bonds broken per second by the Na and Cl ions will be proportional to the concentration c of these ions and to the number of breakable bonds remaining to be broken, that is to $G-g$. The rate of solution can be written $k_1c(G-g)$. Now the rate at which the dissolved g tends to go out of solution will be proportional to g and to a function of c, which just vanishes when $c = C$, the concentration which dissolves all the globulin. The simplest such function is $C-c$, so we write the rate of precipitation as $k_2g(C-c)$. So, for equilibrium,

$$k_2g(C-c) = k_1c(G-g), \quad \text{therefore} \quad p(1-q) = Aq(1-p) = \frac{A}{C}c(1-p), \tag{13}$$

which is identical with (1) and (2). Now A is the ratio of k_1, the velocity of solution of the globulin, to k_2, the velocity of precipitation. If instead of c we use the number of gramme molecules c/M in these equations, its coefficient becomes MA/C, which gives a measure of the ratio of the velocities of solution and precipitation when gramme molecules of different salts are compared as to their actions on globulin of a given concentration. Now the velocity of solution of globulin by a molecule will be proportional to the rate at which the doublets ♯♭ are broken by ionic charges, so it will be proportional to the velocity of the ion and its charge, that is to say its valence. But in the precipitation of globulin the ions have to be extracted from combination with the globulin, so the number of doublets to be broken will be proportional to the valence of the ions. It is assumed here that in salt globulins an ion is loosely attached at the semplar doublet which it breaks, and that as breaking power is proportional to valence, so also is the strength of attachment. The velocity of precipitation will be therefore inversely as the valence. Hence MA/C will be proportional to the square of the valence, which is the modified form given to Mellanby's law in Section 1. As regards the dependence of the velocity of precipitation on velocities, it will arise mostly from motion of the combined ions in the globulin compound. We find, then, that MA/C must be proportional to the velocity of the ions and to a velocity depending on the globulin. Now the velocity of the ion must not be confused with the quantity defined in physics as the ionic velocity, which is a velocity generated under a certain unit external electric force. Apparently, in reacting with globulin, the different ions move with about the same speed. But as regards the effect of change of temperature on the velocity of ions, we know the velocity to be inversely as the viscosity of water at different temperatures. Hence, our equation (3) expresses both this dependence of MA/C on the viscosity of water and on velocity inside the globulin compound. Thus the present theory of colloids is able to give a consistent account of these important features of the chemistry of globulin. The precipitations of globulin hardly need review in this connection, as they have been pretty fully considered in Section 3.

5. *The Electric Conductivity of Globulin Solutions.*

Hardy, by overcoming the experimental difficulties of measuring these conductivities, has obtained data of great importance, which I propose to make more available by expressing them in interpretable formulæ. With HCl 1 gramme of globulin was dissolved in $9{\cdot}32 \times 10^{-5}$ gramme equivalents of acid, and the solutions prepared from this by successive dilution have their strengths given by v, the volume of solution containing 1 gramme equivalent of HCl. From the measured specific conductivity of these solutions the molecular conductivity of the HCl is calculated in the usual way, but it should be noticed that it is expressed with reference to a unit of resistance 10 times as large as that often used, so that conductivities are made 10 times as large. For HCl globulin solutions the molecular conductivity μ of the HCl given by Hardy* is expressed by—

$$1/\mu = 0{\cdot}00026 + 0{\cdot}009v^{-\frac{1}{3}}, \qquad (14)$$

as the following comparison shows:—

Table VI.

v	107	217	434	868	1735	3470
μ calc.	465	568	690	830	991	1170
μ exp.	439	596	706	834	991	1167

At infinite dilution (14) gives $\mu = 3846$, the value for pure HCl being 3840. It appears, then, that at infinite dilution HCl globulin is entirely dissociated, yielding completely ionised HCl. The equation having so extensive a range must have fundamental theoretical significance, which is easily brought out. The occurrence of $v^{-\frac{1}{3}}$ reminds us of Kohlrausch's discovery that the molecular conductivity of dilute electrolytic solutions is a linear function of $v^{-\frac{1}{3}}$, a fact which I have sought to trace back to the 3 occurring in the formula $(H_2O)_3$ for trihydrol.†

For the molecular conductivity of an ion of radius a, valency ν, dielectric capacity K, and charge e, moving through water of dielectric capacity K_0 and viscosity η the formula is—

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* *Loc. cit.*, p. 274.

† See "The Molecular Constitution of Water," and "Ionisation, Ionic Velocities, and Atomic Sizes," 'Phil. Mag.,' [5], vol. 50, and [6], vol. 3.

$$\mu = ve^2K_0/6\pi\eta aK; \tag{15}$$

that is to say, the ionic velocity is inversely as a, so that the time taken by an ion under standard electric force to travel 1 cm. is proportional to a. Now in the case of HCl globulin the corresponding time is proportional to $1/\mu$, and therefore to $0{\cdot}00026+0{\cdot}009v^{-\frac{1}{3}}$. Here 0·00026 represents the time that would be taken by the H and the Cl ions to increase their distance apart by 1 cm., if they were always dissociated from the globulin. The term $0{\cdot}009v^{-\frac{1}{3}}$ represents the time lost on account of the presence of the globulin. The way in which this time is lost is the following: Suppose that HCl globulin is HGCl, yielding two sets of ions HG♯ and Cl♭, and also GCl♭ and H♯. So the H ion travels part of its path as H♯ and part as HG♯, while the Cl ion travels part of its path as Cl♭ and part as GCl♭. Let τ be the fraction of each second which H♯ and Cl♭ spend in the forms HG and GCl. During this fraction each moves with the smaller velocity u due to the large radius of G, for the rest of the second it moves with the ionic velocity u_0 appropriate to H♯ or Cl♭. The average velocity of the ion is $u_0(1-\tau)+u\tau$. Hence the time taken by the H and Cl ions to separate by 1 cm. is proportional to—

$$\frac{1}{u_0(1-\tau)+u\tau} = \frac{1}{u_0}\left(1+\frac{\tau(1-u/u_0)}{1-\tau(1-u/u_0)}\right). \tag{16}$$

If, then, we write $0{\cdot}00026+0{\cdot}009v^{-\frac{1}{3}}$ in the form $0{\cdot}00026\ (1+34{\cdot}6v^{-\frac{1}{3}})$, we have

$$\frac{\tau(1-u/u_0)}{1-\tau(1-u/u_0)} = 34{\cdot}6v^{-\frac{1}{3}}. \tag{17}$$

Denote $\tau(1-u/u_0)$ by x, then we have $x/(1-x)$ proportional to $v^{-\frac{1}{3}}$, and therefore to $c^{\frac{1}{3}}$, where c is the concentration of the HCl. If u/u_0 is small, as Hardy directly proved it to be in the way we shall discuss in the next section, then x stands almost for τ, the fraction of the total H or Cl existing at any moment as HG♯ or GCl♭, while $1-\tau$ stands for the amount of free HCl. In short, τ represents the fraction of the HCl which is combined, and $1-\tau$ that which is free. Hence the proportionality proved above, which may be written

$$x = k(1-x)c^{\frac{1}{3}}, \tag{18}$$

is an equation of chemical equilibrium expressing that the amount of combined HCl existing as HGCl in the form of ions HG and GCl is proportional to the amount of free HCl and to the amount of something else. In "Ionisation, etc."* it is suggested that this something else in ordinary electrolysis is $\sharp H_2O\flat$ broken out of trihydrol. It is shown how the number of these in a solution is proportional to $c^{-\frac{2}{3}}$, so that when they act upon the whole solute c they produce an agent of amount proportional to $c^{-\frac{2}{3}}c$, that is $c^{\frac{1}{3}}$, which, in the present case, acting on the residue $(1-x)$ of free HCl keeps the amount x of combined HCl formed. Hence, for equilibrium, we have $x = k\,(1-x)\,c^{\frac{1}{3}}$ as in (17) and (18). If in (16) we neglect u/u_0 and put $v = 1000$, we find $\tau = 0{\cdot}78$, that is to say, that N/1000 HCl, which is just saturated with globulin, contains 78 per cent. of the H and Cl ions in combination with globulin, and only 22 per cent. free. The experiments of Hardy, when summarised in (14), give a pretty complete insight into the chemistry of HCl globulin.

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* *Loc. cit.*, p. 173.

As regards alkali globulins, the data of Hardy can be expressed by

(1 gramme glob. diss. in $9{\cdot}7 \times 10^{-5}$ gramme equiv. NaOH)
$\mu = 220 + 1/(0{\cdot}0005 + 0{\cdot}031v^{-\frac{1}{3}})$,
(„ „ 18×10^{-5} gramme equiv. NaOH)
$\mu = 290 + 1/(0{\cdot}0005 + 0{\cdot}031v^{-\frac{1}{3}})$,
(glob. diss. in NH_4OH) .... $\mu = 290 + 1/(0{\cdot}00048 + 0{\cdot}023v^{-\frac{1}{3}})$.
(19)

We shall at first discuss only the first and third of these as characteristic of NaOH and NH_4OH. When v is infinite, the first gives $\mu = 2220$, while in the same units the standard data of Kohlrausch would give 444 for Na and 1740 for OH, that is $\mu = 2184$. The formula gives complete dissociation of the alkali globulin at infinite dilution. It is noteworthy that the first term in (18) for NaOH globulin is 220, which is half of the standard value for Na. It appears, then, that half the Na unites with globulin to form a globulinate, which dissociates completely at all the dilutions of Hardy's experiments, giving Na ions, and globulinic acid ions whose velocity is too small to make itself apparent. As the OH of this half of the original NaOH does not contribute to the molecular conductivity in the same way as the Na, but yet appears in the conductivity at infinite dilution, we see that it joins on to the globulin in a similar way to that of the other half of the NaOH. The H of the globulin displaced by the Na atom must also join on to the globulin in a different function. The other half of the NaOH seems to combine with globulin in a way similar to that of HCl. A compound such as NaGOH seems to be formed (with H and OH attached from the first half of the NaOH), yielding ions $NaG\sharp$ and $GOH\flat$ which may be practically two varieties of the globulinic acid with which the other half of the Na combines. From the second of equations (19) it appears that if twice as much NaOH is used as suffices to dissolve all the globulin, its molecular conductivity is but little changed. So in this case a quarter of the base reacts in quite a different way from the remaining three-quarters. The same considerations apply to the case of NH_4OH. The first term 290 is close to the 311 which the standard datum of Kohlrausch would give if half the NH^4 existed as basic ions taking the place of the H ions of globulinic acid. The other half of the NH^4OH combines additively like the second half of the NaOH. In the alkali globulins we must distinguish between the basic half of the alkali and the additive. To the additive half exactly the same considerations apply as to the additive HCl globulinate, and the degree of dissociation of the additive alkali hydrate can be determined as in the case of HCl globulin. Hardy found that to neutralise globulin to phenolphthalein with NaOH the amount of alkali required is twice that needed to dissolve the globulin. Here we have additional evidence that the alkalies act in two ways. Hardy finds that 1 gramme of globulin is dissolved by 10^{-4} gramme equivalents of alkali, and by 2×10^{-4} of acid. Now only half the alkali displaces acidic H, hence the acidic H atoms are only one-quarter the number of places where HCl can add itself to the globulin molecule. Mellanby's measurements on horse globulin do not agree with Hardy's distinction between the number of equivalents of acid and alkali needed to dissolve globulin. He finds a gramme of globulin dissolved by about 10^{-4} gramme equivalents of HCl and about the same number of NaOH. Globulins of different origin and history appear to differ to an extent that limits us at present to a discussion only of orders of magnitude in this connection.

Concerning the molecular conductivity of neutral salt solutions saturated with globulin, Hardy made some measurements. NaCl of concentration 0·16 N had its conductivity depressed 2·4 per cent., of 0·11 N by 2·3 per cent., and of 0·09 N by 2·1 per cent. While of $MgSO_4$ 0·3 N the lowering of conductivity was only 1·4 per cent.

Hardy does not give the strength of the globulin suspension with which he saturated his salt solutions, but states that a gramme of globulin was dissolved by from 10^{-2} gramme equivalents up to 2×10^{-2}. About 100 times as many equivalents of NaCl are required to keep globulin in solution as of NaOH, but as the globulin reduces the conductivity of NaCl about 2 per cent., we may take 2 per cent. of the salt to be combined with globulin, so two equivalents of NaCl actually unite with globulin for one of NaOH. Thus it appears that NaCl in its action on globulin is liker to HCl than to NaOH. The conductivity measurements fully confirm the conclusions arrived at from the solvent powers of neutral salts, namely, that the compounds formed are very unstable and require a large excess of salt to maintain their existence.

6. *The Molecular Mass of Globulin.*

In "A Dynamical Theory of Diffusion for Non-Electrolytes and the Molecular Mass of Albumin,"* I have sought to show how the molecular radius of a molecule can be found from its coefficient of diffusion, having previously obtained the corresponding relation reproduced as (15) in the present paper between molecular conductivity and radius. These two definite and accurately measurable velocities, the ionic and that of diffusion, are convenient aids in the measurement of large molecular masses. From the Graham-Stefan coefficient of diffusion for egg albumin the radius of a gramme molecule of albumin was found to be 30 cm., whence, from the percentage composition, the following formula is obtained for albumin, $C_{1436}H_{2364}N_{359}O_{482}S_{15}$ with a molecular mass 32,814. Now Hardy has obtained a direct measurement of the ionic velocity of globulin, namely, about 10^{-4} cm. per sec., which corresponds to a molecular or rather ionic conductivity 100 in the units used by Hardy, and 10 in the units of Kohlrausch. With the latter units (15) is transformed† to

$$B^{\frac{1}{3}} = 280\nu/\mu K, \tag{20}$$

in which K is the dielectric capacity of the ion, having for globulin the value 2 nearly, because for proteids the index of refraction which equals $K^{\frac{1}{2}}$ is about 1·4. B is the volume of the molecules in a gramme molecule of the ion, and ν its valence. We are going to use this equation to find B for globulin, using Hardy's value 10 for μ. But so far ν is unknown. It is the basicity of globulinic acid which is easily determined approximately by the facts we have in hand. Thus it has been shown that when NaOH dissolves globulin, half of it acts as a relatively strong base. Let M be the molecular mass of globulin, then ν gramme molecules of NaOH act as a strong base with M grammes of globulin. But Hardy finds 10^{-4} gramme molecule of NaOH just dissolves 1 gramme of globulin, so $10^{-4}/2$ gramme molecule satisfies the ν-basicity of globulin

$$2 \times 10^4\nu = M. \tag{21}$$

With $\mu = 10$ equation (20) gives

$$B^{\frac{1}{3}} = 14\nu. \tag{22}$$

For the percentage composition of globulin, we have Hammarsten's determinations, with which I shall give the limiting volume of a gramme atom of the elements taken from my 'Phil. Mag.' papers on molecular force.

	C.	H.	N.	O.	S.
Per cent..........	52·71	7·01	15·85	23·32	1·11
Atomic B	8	4	8	6	18

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* 'Phil. Mag.,' [6], vol. 9, 1905.

† See "The Dielectric Capacity of Atoms," 'Phil. Mag.,' [6], vol. 7.

Let x be the number of atoms of C in the molecule of globulin, then

$$12x = 0{\cdot}5271\text{M}, \tag{23}$$

and also

$$x\left\{8+\frac{12}{52{\cdot}71}\left(4\,.\,\frac{7{\cdot}01}{1}+8\,.\,\frac{15{\cdot}85}{14}+6\,.\,\frac{23{\cdot}32}{16}+18\,.\,\frac{1{\cdot}11}{32}\right)\right\} = \text{B}. \tag{24}$$

These four equations (21, 22, 23, 24) give us the four unknowns ν, x, B, and M. The best way of solving them is to give ν integral values, and find which value makes the four equations as consistent as possible within permissible limits of error. With $\nu = 1$ (21) gives M = 20,000, and then (22) gives B = 2744, which in (24) yields $x = 147{\cdot}7$, and this in (23) makes M = 3362. As they stand, the two values of M, namely 20,000 and 3362, are inconsistent. If $\nu = 2$, the first becomes 40,000 and the second 8×3362 or 26,896. For $\nu = 3$ the comparison is between 60,000 and 3362×27 or 90,774. No value of ν above 3 is permissible. Hence the choice for the formula of globulin is between one which makes it a dibasic acid of molecular mass about 40,000 and another which makes it a tribasic acid of molecular mass about 60,000. Let us inquire what changes in the experimental data would make the four equations strictly consistent for $\nu = 2$ and $\nu = 3$. The most uncertain number is Hardy's valuable datum $\mu = 10$. For a transparent opalescent dialysed solution of NaOH globulin he found $\mu = 7{\cdot}66$ and estimated 10 as the probable limit to which the value would rise with complete ionisation of the compound. But his solution being opalescent, there must have been some electric endosmose such as he found with $\text{H}\bar{\text{A}}$ globulin, for which it raised μ up to 23. In the observed 7·66 for NaOH globulin there is already some compensation of errors, and it is an open question whether the limit is more or less than 10. It is easy to calculate the value of μ in (20) which would make $\nu = 2$ and M = 40,000 satisfy (21, 23, 24). It is 8·76. So for $\nu = 3$ and M = 60,000 we should need $\mu = 11{\cdot}50$. Hence the decision between the two alternatives should be reached by a sharper determination of the ionic velocity of globulin or by its coefficient of diffusion, or preferably by both of these methods. Probably 8·76 is the better value for μ. With this and $\nu = 2$ and M = 40,000 we get $x = 1756$. Hence the formula of globulin is $C_{1756}H_{2804}N_{452}O_{584}S_{14}$.

This formula of the globulin molecule furnishes a molecular mass 1·22 times that given above for egg albumin obtained from the Graham-Stefan coefficient of diffusion. That formula can be greatly simplified for discussion if we replace the number of carbon atoms 1436 by the round number 1440, the 2364 of H by 2400, the 359 of N by 360, and the 482 of O by 480, and neglect for a while the 15 for S. These changes keep the percentage composition of egg albumin true within the limits of analytical error, and enable us to write the main part of the egg albumin molecule as 120 ($C_{12}H_{20}N_3O_4$).

The formula of globulin cannot be quite so much simplified, but we can write it $146(C_{12}H_{19}N_3O_4)+14N$, neglecting S meanwhile. The group $C_{12}H_{20}N_3O_4$, which is repeated 120 times in the albumin molecule, is closely related on the one hand to the polypeptides synthesised by E. Fischer, and to the pure peptones prepared by Siegfried. For the derivation of dipeptides and tripeptides from the amino acids by abstraction of water we have the equations*

$$2NH_2(CH_2)_nCOOH - H_2O = NH_2(CH_2)_{2n}NHCOCOOH, \text{ dipeptide};$$
$$3NH_2(CH_2)_nCOOH - 2H_2O = NH_2(CH_2)_{3n}(NHCO)_2COOH, \text{ tripeptide.}$$

If, now, in this general formula for a tripeptide we put $n = 3$, we obtain $C_{12}H_{23}N_3O_4$, a group containing 3H more than our typical albumin group above. If, then, we remove 3H from this polypeptide, we obtain a trivalent radicle, $C_{12}H_{20}N_3O_4$, which we find repeated 120 times in the molecule of egg albumin. It hardly matters at present how many times CH_2 may be removed from one of these groups and inserted into others, for we are considering only the broad plan on which the albumin molecule is built.

Similarly, the properties of globulin depend on a certain preponderating group whose average composition is close to $C_{12}H_{19}N_3O_4$. These groups are almost as closely related to the pure natural peptones prepared by Siegfried as to the synthesised polypeptides of E. Fischer.† These are the antipeptones α and β, or trypsin-fibrin peptones $C_{10}H_{17}N_3O_5$ and $C_{11}H_{19}N_3O_5$, of which the next homologue would be $C_{12}H_{21}N_3O_5$, differing from our albumin group by OH and from our globulin group by OH_2. Even if these formulæ need to be doubled, the relation would not be altered. Then come the pepsin-fibrin peptones α and β, $C_{21}H_{34}N_6O_9$, $C_{21}H_{36}N_6O_{10}$. It is significant that the latter could be produced by the additive union of the antipeptones α and β.

Now, the experiments of W. Neumann and Siegfried have yielded the result that in the antipeptones the H ions are to the OH ions as 2 : 1, and in the pepsin peptones as 3 : 2. But the number of H ions gives the number of COOH radicles in the molecule, while that of the OH ions gives the number of NH_2 radicles. Hence we infer that the group $C_{12}H_{19}N_3O_4$, when converted into an antipeptone, contains COOH twice and NH_2 once. Hence, in the joining up of such groups to the number of 146 to form globulin, there must be a large amount of internal salt formation, since COOH occurs only two or three times in the complete globulin molecule. It is most probable that the internal salt formation goes on in such a way that COOH and NH_2 neutralise one another.* Thus in the extreme case of all the oxygen existing as COO in $C_{12}H_{19}N_3O_4$, two-thirds of the total nitrogen would be present as NH_2 joined up with COOH in the globulin molecule. When the globulin molecule is made an ion, it has probably only two COO radicles showing the acid character. Hence we infer from our discussion of Hardy's conductivity measurements that the globulin molecule contains NH_2 eight times in such a form that HCl can join on as it does with NH_3 to form NH_4Cl. These eight NH_2 and the two COOH are the only ones left free out of about 290 of each sort existing in the globulin molecule. By more or less drastic treatment the latent NH_2 and COOH radicles may be called into partial action, and so globulin and albumin may be made to show apparently higher acidity and basicity than the eight and the two determined above. In the same way arise the discrepant results of different experimenters, discussed in Section 3, as to the percentage of heavy metals in their compounds with albumins. Different treatments open out or break up the albumin molecule differently. But yet a rather striking agreement appears in these discordant results if we regard them in the following way. The range in the percentages of Cu in albuminates and globulinates is 0·45 in Mellanby's globulinate, 0·71 in the albuminate of Chittenden and Whitehouse, 1·2 Mörner, 1·35 Harnack, 1·85 Mörner, 2·19 Chittenden and Whitehouse, 2·64 Harnack, and 11, the average of Ritthausen's measurements with vegetable albumins. For the weight of proteid combined with a gramme equivalent of Cu (31·6) these give the numbers—

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* See Cohnheim's 'Chemie der Eiweisskörper,' p. 59.

† Siegfried, 'Ber. d. Deutsch. chem. Ges.,' vol. 33; 'Zeitsch. f. Physiol. Chem.,' vols. 35 38, 45.

7020	4450	2630	2340	1710	1440	1200	287
24·1	15·3	9·05	8·04	5·87	4·96	4·10	0·987

which are expressed in the second row as multiples of 291, these multiples being all nearly exact integers 1, 4, 5, 6, 8, 9, 15, and 24. Here, then, we have evidence of the splitting up or opening out of the albumin molecule into parts, each of which contains an integral number of a group whose molecular mass is about 291, which is quite close to the 270 of our $C_{12}H_{20}N_3O_4$. This group with H_2O would give 288. This seems a fairly conclusive proof that there is a large amount of internal salt formation in albumins. The 3300 found in Section 3 for the mean mass of albumin combined with an equivalent of heavy metal in Chittenden and Whitehouse's experiments represents about 12 of our groups. It is interesting to note that the treatment of a vegetable albumin with copper breaks up the albumin molecule into parts of about the size of a peptone molecule. This probably accounts for the powerful toxicity of copper towards those lower plants to whose protoplasm it gains ready access.

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* See Mann, 'Chemistry of the Proteids,' pp. 210 and 211.

The large molecular mass of about 40,000 found for globulin accounts for the fact emphasised by Hardy that the polybasic acids H_2SO_4, H_3PO_4, combine by molecules and not by equivalents with globulin. The eight NH_2 radicles are so widely distributed over the large molecule that only one can come within the reach of an acid molecule. Thus only one OH radicle in H_2SO_4 and H_3PO_4 can operate. In the same way, since globulin contains only two COOH radicles, these are probably far apart, and a divalent atom like Ba can replace only one H. It would be possible for Ba to unite two globulin molecules through a COO radicle in each, and for SO_4 to unite two through an NH_2 radicle in each. Probably the colloidal state of a globulin suspension is due to the ordinary ineffective doublet of trivalent nitrogen becoming effective, thus enabling the globulin molecules to grip each other by means of the eight free NH_2 radicles in each. Solution in acid occurs when the acid satisfies the extra valencies of the NH_2 radicles, thus breaking their hold on one another. The semplar G of globulin escapes into the freedom of solution as part of the molecule $G(HCl)_8$. In edestin, T. B. Osborne has found what he calls the monohydrochloride insoluble, while the dihydrochloride is soluble. The explanation of this is that in the monohydrochloride sufficient junctions amongst the semplars are still left intact to keep the colloidal monohydrochloride from going into solution. The equivalent weights, 14,300 to 7000 given by Osborne for edestin, are 1/3 and 1/6 of the 40,000 found above for globulin.

As to solution of colloidal globulin in bases, we have seen that in the case of Hardy's ox globulin it was not sufficient to supply merely enough NaOH to form the easily dissociable globulin salt, but twice as much was required for complete solution. Here the extra NaOH is required along with OH and H due to the first half of the NaOH to break the colloidal junctions between the semplars. How it does this as efficiently as four times the equivalent HCl forms an interesting chemical question. The probable mode of operation of neutral salts in dissolving colloidal globulin has already been discussed.

Summary.

By expressing the experimental results of Hardy and Mellanby in simple formulæ, it is shown that the solution of globulin and its precipitation take place under simple conditions of chemical equilibrium. For example, if p is the fraction of a globulin suspension dissolved in a salt solution whose concentration is the fraction q of C that is required just to dissolve the whole

of the suspension, we get equation (1) $p(1-q) = Aq(1-p)$, in which A is the ratio of a velocity of solution to a velocity of precipitation. Mellanby's discovery of the dependence of M/C on valency and ionic velocity is applied to MA/C, M being molecular mass, and it is shown that when temperature varies, not only does MA/C depend upon the viscosity of the solvent water, but also on a function of temperature given in equation (3) which expresses the part played by globulin. It is noteworthy that this function has a minimum value about 40° C., near the temperature of warm-blooded animals.

For the precipitation of globulin by excess of $(NH_4)_2SO_4$, equation (5) is established, namely $p(1+p) = 28{\cdot}8\,(c - 0{\cdot}152)$, p being the fraction which the precipitated globulin is of the whole and c the concentration of the $(NH_4)_2SO_4$ solution in grammes per cubic centimetre.

Then follow formulæ for the remarkable precipitation of globulin by acids from solution in neutral salts. From these it appears that three compounds of globulin react in producing this precipitate.

Section 4 is devoted to a theory of the colloidal state, namely, that a colloid consists of molecules which are chemically united neighbour to neighbour by the action of valencies which are usually latent. Cases of multiple valency like that of nitrogen and oxygen are best accounted for by the electron theory of valence due to Helmholtz, with the assumption that a single atom can contain both negative and positive electrons. Thus the nitrogen atom has four negative electrons and one positive. In trivalent nitrogen the positive electron neutralises one of the negative, so that the atom has only three effective negative electrons along with an ineffective doublet. But if this doublet is opened out, it can link on its atom to two neighbour atoms, the negative electron grasping a positive, and the positive a negative.

According to this chemical theory of the colloidal state, the term molecule ceases to have a useful meaning when applied to a colloid, so the term semplar is used to name that structure which is repeated like a pattern in three dimensions through a colloid. By suppression of the colloid-producing valencies of doublets, a mass of semplars is caused to fall into a collection of separate molecules. In illustration of the usefulness of this theory, it is applied to show the dependence of the coagulating power of an ion on its valence. It is then applied also to explain the remarkable fact that the amount of globulin dissolved by a given salt solution from a globulin suspension depends on the concentration of the suspension. The action of the ions of a neutral salt in dissolving globulin is treated as only another manifestation of the same electrical effect which enables them to coagulate arsenious sulphide. This theory of the colloidal state leads to a theory of equation (1) and of the laws of A in it, especially that MA/C is proportional to the sum of the squares of the valencies of the ions of the electrolyte.

In Section 5 the experiments of Hardy on the conductivities of globulin solutions are expressed by formulæ which admit of very simple interpretation, and connect these conductivities with those of ordinary electrolytes. At infinite dilution the HCl compound of globulin is completely dissociated, its conductivity being that due to the completely ionised HCl, the part due to the globulin being then too small to appear. For any other dilution the amounts of combined and free HCl can be calculated by equations (17) and (18). In the compounds of NaOH and NH_4OH with globulin the conductivities show that half of the base combines with the globulin in a way which proves it to be an acid, the resulting salt being completely dissociated and showing the conductivity due to Na or NH_4 alone, that of the globulinic acid being too small to appear. The other half of the base which is required to dissolve globulin completely combines with it in a way similar to that of HCl, namely, by addition.

In Section 6 globulin is shown to have probably a molecular mass 40,000 and a basicity 2, the alternative being a mass 60,000 and basicity 3. Further experiments like those of Hardy on the ionic velocity of globulin and also on the coefficient of diffusion of globulin solutions would decide the matter, though doubtless various globulins differ in mass and basicity. A group, $C_{12}H_{20}N_3O_4$, related to polypeptides and peptones is shown to be the predominant structure in albumins. The discrepant results of different experimenters on the precipitation of albumin by heavy metals fall into harmony when it is proved that they precipitated different integral numbers of a group such as this in combination with an equivalent of heavy metal.

Observations on the Life-history of Adelea ovata, *Aimé Schneider, with a Note on a New Gregarine from the Gut of* Lithobius forficatus.

By C. C. Dobell, B.A., Scholar of Trinity College, Cambridge.

(Communicated by J. J. Lister, F.R.S. Received November 19, 1906,—
Read January 17, 1907.)

[Plates 2 and 3.]

At the suggestion of Mr. J. J. Lister, F.R.S., I recently undertook a reinvestigation of the sporozoan parasites occurring in the gut of the centipede, *Lithobius forficatus.* The present paper is the result of these investigations. It is with pleasure that I take this opportunity of expressing my thanks for the advice and assistance I have received not only from Mr. Lister, but also from Mr. Adam Sedgwick, F.R.S., and from Mr. W. S. Perrin.

No less than six protozoan parasites have been recorded from the gut of *Lithobius forficatus*—four Coccidia and two Gregarines. The Cambridge centipede appears to harbour but a single one of the former—namely, *Adelea ovata,* A. Schn.—whilst both the latter* have been observed in rather less than 1 per cent. of the animals examined. It is with the sexual phases of the life cycle of *A. ovata* that the present paper is chiefly concerned.

Previous Work.—*Adelea ovata* was first described† by Aimé Schneider in 1875 [12], and although a part of the sexual process was observed, it was not recognised as such. Schneider published some further particulars in 1892 [14]. In 1897 Schaudinn and Siedlecki‡ published a preliminary account of the Coccidia of the centipede, and later—in 1899—Siedlecki [16] gave a detailed description of the whole life-history of *Adelea ovata.* Subsequent writers have described other species of the genus *Adelea,* and in all cases the life cycle appears to correspond closely with that recorded by Siedlecki. I may say that I am able to confirm most of the results obtained by this writer, but I have come to different conclusions regarding one or two points. The most important of these deals with the formation of the microgametes.

Methods.—The best results have been obtained by adopting Schaudinn's methods [11]. The entire gut was removed, and the epithelial cells and gut-contents spread out upon a coverslip. The films thus obtained were instantly fixed by immersion in hot sublimate-alcohol containing a trace of acetic acid (Schaudinn). After fixation the films were treated with alcohol containing iodine, and stained in Bütschli's modification of Delafield's hæmatoxylin.* This I prepare by adding 1-per-cent. acetic acid solution to a 0·5-per-cent. solution of the ordinary concentrated Delafield's hæmatoxylin in water until a pink colour is produced. Staining, for all stages except spores, is complete in from 15 to 30 hours.

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* Viz., *Actinocephalus dujardini,* A. Schn., and *Echinomera hispida,* A. Schn.

† As a gregarine.

‡ Schaudinn and Siedlecki, 'Sitz. ber. d. d. zool. Gesell.,' 1897.

Giemsa's modification of the Romanowsky-Nocht stain was tried, among others, but it proved to be of little use for delicate nuclear structures, owing to the drying which necessarily takes place.

Transverse sections of the gut were made, but they were found to be unsatisfactory. Moist films are far more useful for examining the Coccidia.

I will now proceed to describe the stages which I have observed and which differ from those hitherto recorded.

(1) ASSOCIATION.—The entire genus *Adelea* is characterised by a precocious association of the gametocytes. This was observed in *A. ovata* as long ago as 1875, although, strangely enough, it was not even suspected of being connected with any sexual process. Association, it may be noted, takes place in the lumen of the gut, and not in the epithelium. Siedlecki, who ultimately interpreted this phase correctly, states that but a single microgametocyte ever attaches itself to a macrogametocyte. Only once did he observe two microgametocytes attached to the same female individual. My observations, on the contrary, lead me to believe that this condition is not uncommon. I have seen upwards of a dozen instances in which two microgametocytes were attached to the same macrogametocyte. Apparently, these microgametocytes do not always develop or become attached simultaneously, for they may be found in different stages of maturity (*cf.* Plate 3, fig. 3). Association in which more than one microgametocyte takes part has been described by Laveran† in the closely allied form *Klossia helicina.*

An association of two microgametocytes with one another as described in *A. mesnili* (Pérez) and *A. zonula* (Moroff), has never come under my observation.

(2) FORMATION OF MICROGAMETES.—The only detailed account of the genesis of the male gametes of *A. ovata* is that given by Siedlecki. He states that the nucleus of the microgametocyte divides into two daughter-nuclei, each of which then divides in a plane at right angles to that of the first division, so that four nuclei result. These nuclei then develop into the

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* Bütschli, 'Zeitschr. f. wiss. Mik.,' ix, 2, 1892, p. 197.

† Laveran, 'C. R. Soc. Biol. Paris,' 1898 (Novembre).

four microgametes, one of which is destined to fertilise the macrogamete; the remaining three perish. In the first nuclear division it was believed that the quantity of chromatin was reduced, whereas the number of chromosomes was reduced in the second division. I am convinced that this is not a correct interpretation of this particular gametogenesis; and the inference that the maturation of these gametes approaches the metazoan type is, to my mind, quite unjustifiable.

To follow the formation of the male gametes from the beginning, it is necessary to describe in detail a merozoite of the male type. Such a merozoite is shown in the accompanying figure (text-fig. 1).

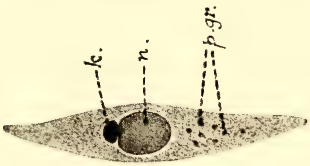

FIG. 1.

It is seen to consist of a somewhat fusiform (or sometimes falciform) body, containing a nucleus (*n.*) and a large peripheral karyosome (*k.*). Sometimes two or even three karyosomes are present, but the usual number is one. Occasionally it appears to be extranuclear. At one end of the merozoite—usually that remote from the karyosome—a number of granules is usually, though not always, present. These are the so-called "pigment granules" (*p. gr.*). I am inclined to believe that they are really chromatin granules. They stain deeply with hæmatoxylin, and are coloured red by Giemsa's stain. They are never present in macromerozoites.

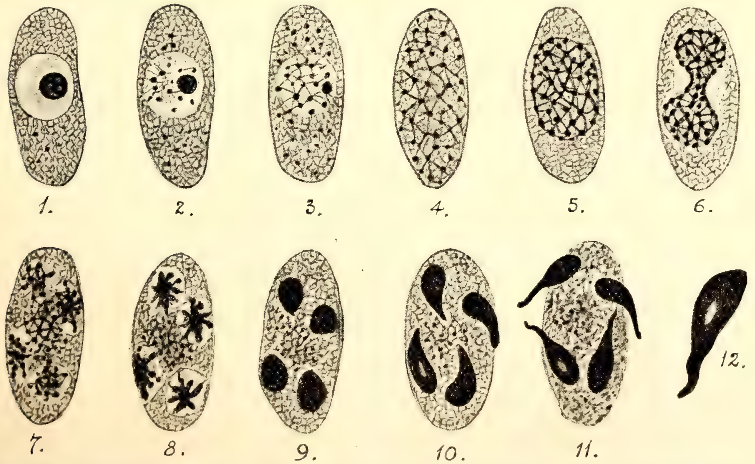

FIG. 2.—Semi-diagrammatic Representation of Formation of Microgametes. The macrogametocyte is not shown.

A micromerozoite, such as that just described, may increase in size and become a microgametocyte (text-fig. 2 (1)). It then becomes attached to a macrogametocyte in the usual manner. During its transformation into a microgametocyte, the karyosome travels to the centre of the nucleus, and is apparently reinforced by the nuclear chromatin. A part of this latter, however, is usually to be found lying free in the clear nucleus in the form of granules.

The next step towards the formation of the microgametes consists in a dispersal of the karyosome and the formation of a chromatic network in the nucleus. This is shown in (2) text-fig. 2, and may take place before association occurs. Further breaking up* of the karyosome now ensues—provided that association has taken place—the chromatin particles invading the cytoplasm and there forming a chromidial† net (3). When the karyosome is completely broken up, and the outline of the nucleus has completely disappeared, the microgametocyte is seen to contain a chromidial net completely filling it from end to end (4). The foregoing stages (1 to 4) may be conveniently termed the *analytic* phase. Those which follow constitute what I may call the *synthetic* phase, in which four new nuclei are built up from the chromatic elements of the old nucleus. Synthesis begins with the gathering up of the chromidial net into a more or less rounded mass (5) which soon becomes constricted in the middle, giving rise to a dumb-bell shaped reticulum (6). If deeply stained, it has the appearance of a dividing nucleus.‡ The ends of the dumb-bell now extend transversely, and the chromatin becomes localised at the four poles so formed (7). At the same time, the intermediate portion becomes diffuse; it finally breaks up, undergoes degeneration, and is left behind—with a small quantity of pigment—in the residuum of the microgametocyte when the microgametes forsake it.

Four special points, we have seen, have now become centres for the aggregation of chromatin. Chromatin continues to travel to each point, and at first arranges itself in the form of a loose net. The net then resolves itself into a stellate mass of chromatin. Hence the highly-characteristic figure of a microgametocyte containing four star-shaped nuclei is presented (8). The stars finally become rounded off (9)—the spherical nuclei so formed afterwards elongating to form the microgametes, which apparently consist entirely of chromatin ("chromatozoites") (10—11). Many of the microgametes enclose a clear space resembling the vacuole described by Schaudinn in *Coccidium schubergi.* A mature microgamete is shown on a larger scale in text-fig. 2 (12).

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* The exact manner in which the breaking up of the karyosome takes place is unknown to me. I have never been able to watch the process in the living animal. That breaking up does occur is inferred from the fact that the karyosome becomes smaller and smaller, whilst the chromatin granules increase in number.

† See, further, under "Chromidia," next page.

‡ I should like to mention here the fact that published figures purporting to show two nuclei in the microgametocyte really show what I believe to be the stage just described. All the figures given by Siedlecki show two "nuclei" connected together—in no case are they completely separate. Siedlecki's "corpuscule intermédiaire" is, I believe, a more deeply stained portion of the intervening network.

It will be seen from the foregoing that my account of the formation of the male gametes differs considerably from that of previous writers.

It now becomes an interesting point to determine whether a similar chromidial condition occurs at any period in the life-history of the female forms. So far, I have been unable to reach any satisfactory conclusion with regard to this. However, the following observations seem worth recording in this connection :—

(*a*) A single instance has occurred in which the individuals of a group of macromerozoites were found to have their nuclei in a chromidial condition (see Plate 3, fig. 6).

(*b*) Since the stellate nuclei of the microgametocyte are formed by synthesis from chromidia, it is possible that the stellate nuclei of the macroschizont may have a similar origin. Although, up to the present, I have not been able to demonstrate that this is the case, nevertheless, I think that the macroschizont, of which fig. 5, Plate 3, is an exact copy, probably shows a stage in which neuclei are being synthesised by the concentration of chromidia at various points. It should be noticed that a considerable quantity of chromatin is scattered through the cytoplasm. I am, of course, ready to admit that this may be quite a wrong interpretation of the appearances. Possibly they are due to degenerative changes. Nevertheless, the phenomenon of chromidia-formation has assumed such importance, owing to recent work in other groups of the Protozoa, that I believe the conditions described may be found to be not altogether insignificant.

Chromidia.—So many new facts regarding chromidial structures have recently come to light, and so little notice has been taken of them in England, that I think a few words on chromidia in general may not be out of place here. As it is obviously impossible to treat the subject fully in the present paper, I have given a short list of papers bearing on the subject in the literature list (B) at the end; for a more detailed list of references I refer the reader to the writings of Hertwig [21] and Goldschmidt [18, 19].

The conception of chromidia was first formulated by Richard Hertwig in 1902 [20], and resulted from his observations on *Actinosphærium, Arcella,* etc.

Since then, many other "chromidial" nuclear phenomena have been described. Indeed, it is now a matter of some difficulty to give a definition of chromidia which will embrace all conditions so described. In its widest sense, the term is applied to all chromatin substance (*i.e.*, all matter which takes up nuclear stains) which lies within the cell and which is not gathered together in the form of a definite nucleus. Chromatin granules of diverse shapes and sizes, strands, networks—all these come in this category; and, moreover, the physiology of these structures seems to be no less heterogeneous than their morphology. For chromidia seem to be formed sometimes as the result of degeneration, sometimes as a form of nuclear division, sometimes as katabolic products of nuclear activity—sometimes (as in bacteria), being the normal vegetative stage of the neucleus—and so on.

Many new terms have been introduced for these various forms of chromidia. Since many of these have not received general acceptance, and since many are, I think, quite unjustifiable, owing to our present ignorance of the causes underlying the different phenomena, I have used but two terms in my description of the nuclear changes in *A. ovata*,—"*chromidia*" for separate chromatin granules lying in the cytoplasm, and "*chromidial net*" for a collection of chromatin granules united by a reticulum. Amongst the terms which are more frequently met with in papers on this subject the following may be mentioned:—The name "*Sporetium*" (Goldschmidt) has been given to chromidial structures formed previous to gamete formation. "*Protogonoplasm*" (Calkins) and "*Idiochromidium*" (Goldschmidt) are other terms applied to the same structures. Then, again, we have such terms as "*Trophochromidium*" and "*Chromidium*" (*sensu stricto*) applied by Goldschmidt to chromidial structures of a vegetative (as opposed to reproductive) nature. Other terms, such as "distributed nucleus" (Calkins), etc., require no special explanation.

A chromidial condition of the nucleus may occur, apparently, not only in all the chief subdivisions of the Protozoa, but also in some Metazoa and in Bacteria.

The great interest which attaches to chromidia arises from the fact that investigation of these structures has revealed a "Doppelkernigkeit," or nuclear dimorphism, in many Protozoa. That is to say, two distinct elements can be recognised in many protozoan nuclei—one, a "*propagative*" element, the other, a "*somatic*." The propagative seems specially liable to separate from the somatic as chromidia (*cf.* Schaudinn, Neresheimer, etc.). The heterokaryote condition of the Ciliata thus finds a parallel in many other Protozoa. To the single nucleus, combining the chromatic elements proper both to the soma and reproduction, is given the name "*amphinucleus*."

From the foregoing remarks, I think it will be evident that the subject of chromidia is one of great importance, but not one on which we are at present, owing to our lack of knowledge, able to dogmatise. I will conclude by quoting Mesnil. "Il faut," says this writer, "bien se garder de trop généraliser, et chaque cas où le protoplasme se charge de granulations chromatiques doit être étudié avec soin à ce nouveau point de vue."

Note on a New Gregarine from the Gut of Lithobius forficatus.

Whilst examining centipedes for stages in the life-history of *A. ovata*, I came across the trophozoites of a gregarine which, I think, has not been recorded hitherto. The trophozoites were very numerous, but no spores or other stages could be found. Hence it is at present impossible to refer the animal to its proper systematic position.

Only one centipede which I examined contained these gregarines, but in this particular case they were very numerous. The Lithobius infected was in a dying condition just before I examined it, possibly owing to the harmful effects of the parasite.

The gregarine has an exceedingly simple structure (see fig. 3). There is no epimerite, and the body is non-septate and usually slightly more pointed at one end than at the other. A large nucleus containing a big karyosome is situated more or less in the middle of the animal: its position varies. The average length is 127 μ, breadth 48 μ. The measurements were made from permanent preparations.

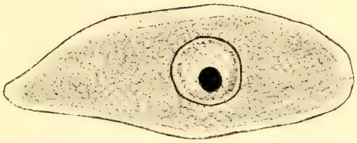


FIG. 3.

LITERATURE.

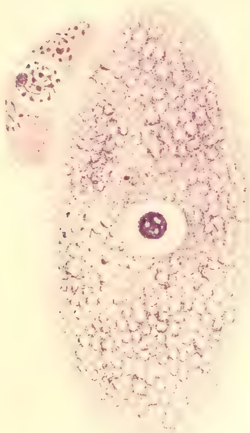
(*A.*) *Relating to* Adelea.

1. Bütschli, O. "Kleine Beiträge zur Kenntniss der Gregarinen: III, Über eine eiförmige Psorospermie aus dem Darm des *Lithobius forficatus*" ('Zeitschr. f. wiss. Zool.,' vol. 35, 1881).
2. Doflein, F. "Die Protozoen als Parasiten u. Krankheitserreger, etc.," Jena, 1901.
3. Labbé, A. "Recherches zoologiques, cytologiques et biologiques sur les Coccidies" ('Arch. de Zool. exp. et gén.,' vol. 4, 1896).
4 ——— Art. "Sporozoa," in 'Das Tierreich,' Lief. 5, 1899.

5. Léger, L. "Sur la morphologie et le developpement des Microgamètes des Coccidies" ('Arch. de Zool. exp. et gén., Notes et Revue,' vol. 6, 1898).
6. ——— "Essai sur la Classification des Coccidies et description de quelques espèces nouvelles ou peu connues" ('Ann. de Mus. de Hist. Nat. Marseille—Bulletin,' vol. 1, fasc. 1, 1898).
7. ——— "Sporozoaires parasites de l'*Embia solieri*, Rambur" ('Arch. f. Protistenk.,' vol. 3, 1904).
8. Minchin, E. A. Art. "Sporozoa," in Lankester's 'Treatise on Zoology,' Part I, fasc. 2, 1903.
9. Moroff, T. "Untersuchung über Coccidien. I. *Adelea zonula*, nov. sp." ('Arch. f. Protistenk.,' vol. 8, pt. 1, 1906).
10. Pérez, C. "Le cycle évolutif de l'*Adelea mesnili*, coccidie cœlomique, parasite d'un Lepidoptère" ('Arch. f. Protistenk.,' vol. 2, 1903).
11. Schaudinn, F. "Untersuchungen über den Generationswechsel bei Coccidien" ('Zool. Jahrb., Abth. f. Anat.,' vol. 13, 1900).
12. Schneider, Aimé. "Contributions à l'histoire des Grégarines des invertèbres de Paris et de Roscoff" ('Arch. de Zool. exp. et gén.,' vol. 4, 1875).
13. ——— "Coccidies nouvelles ou peu connues" ('Tabl. Zool.,' vol. 1, 1880).
14. ——— "Coccidies nouvelles, etc." ('Tabl. Zool.,' vol. 2, 1892).
15. Siedlecki, M. "Étude cytologique et cycle évolutif de la Coccidie de la Seiche" ('Ann. de l'Inst. Past.,' vol. 12, 1898).
16. ——— "Étude cytologique et cycle évolutif de *Adelea ovata*, Schneider" ('Ann. de l'Inst. Past.,' vol. 13, 1899).

(*B.*) *Relating to Chromidia.*

17. Calkins, G. N. "Evidences of a Sexual-cycle in the Life-history of *Amœba proteus*" ('Arch. f. Protistenk.,' vol. 5, 1905).
18. Goldschmidt, R. "Der Chromidialapparat lebhaft funktionierender Gewebszellen" ('Zool. Jahrb., Abth. f. Anat.,' vol. 21, 1904).
19. ——— "Die Chromidien der Protozoen" ('Arch. f. Protistenk.,' vol. 5, 1905).
20. Gonder, R. "Beiträge zur Kenntnis d. Kernverhältnisse bei den in Cephalopoden schmarotzenden Infusorien" ('Arch. f. Protistenk.,' vol. 5, 1905).
21. Hertwig, R. "Die Protozoen und die Zelltheorie" ('Arch. f. Protistenk.,' vol. 1, 1902).
22. ——— "Über physiologische Degeneration bei *Actinosphærium eichhorni*" ('Festschr. f. E. Häckel,' 1904).
23. Lister, J. J. "The Life-history of the Foraminifera" ('Presidential Address, Sec. D, Brit. Assoc.,' 1906).
24. Mesnil, F. "Chromidies et questions connexes" ('Bull. Inst. Pasteur,' vol. 3, 1905).
25. Neresheimer, E. "Die Zeugungskreis v. Opalina" ('Sitzungsb. d. Gesell. f. Morph. u. Physiol. in München,' Part 1, 1906).
26. Schaudinn, F. "Beitrag zur Kenntnis der Bakterien u. verwandter Organismen"—1. *Bacterium butschlii* ('Arch. f. Protistenk.,' vol. 1, 1902); 2. *Bacillus sporonema* (*loc. cit.*, vol. 2, 1903).
27. ——— "Untersuchung über d. Fortpflanzung einiger Rhizopoden" ('Arb. aus d. k. Gesundheitsamte,' vol. 19, pt. 3, 1903).
28. Zülzer, M. "Beitrag zur Kenntnis von *Difflugia urceolata*, Carter" ('Arch. f. Protistenk.,' vol. 4, 1904).



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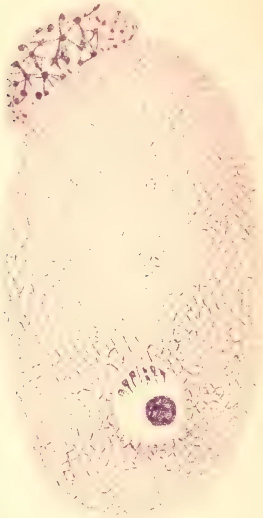

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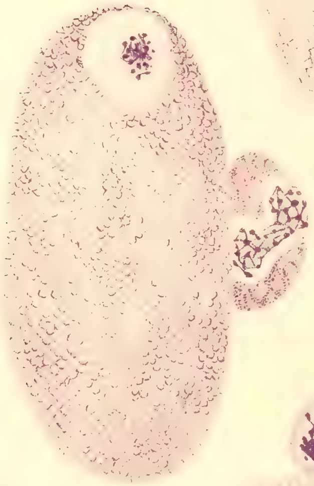

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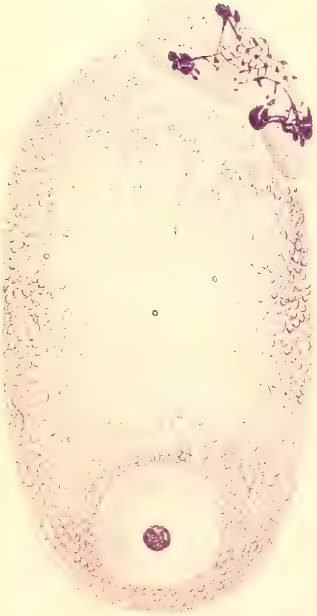

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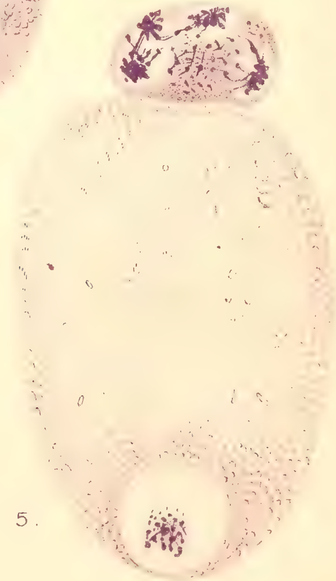

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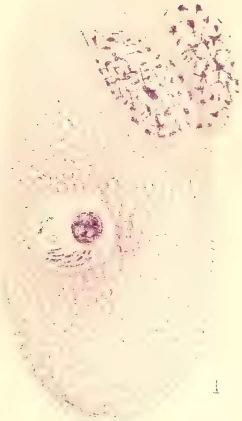

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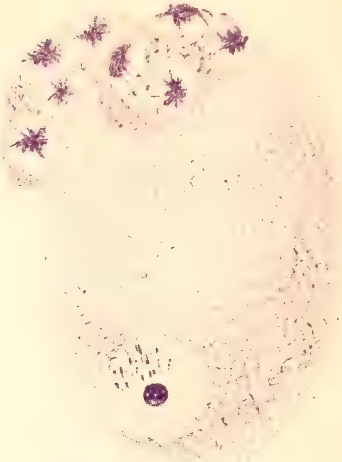

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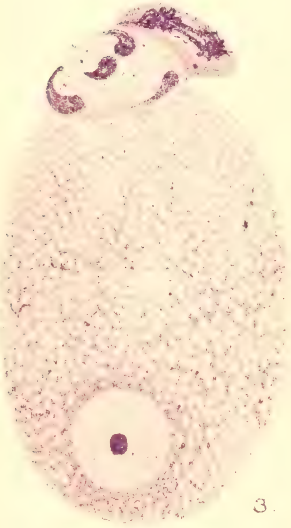

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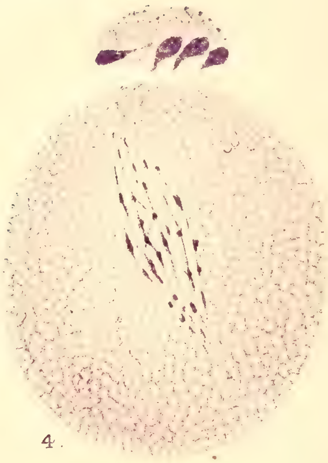

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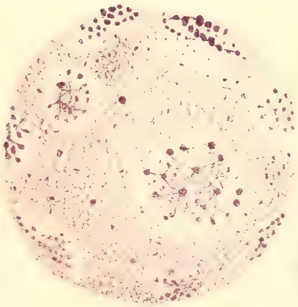

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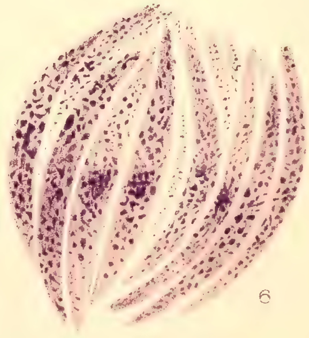

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Huth Lith. London.

DESCRIPTION OF PLATES.

[All figures are drawn from permanent preparations stained with hæmatoxylin. Drawings made under a 3-mm. apochromatic objective (Zeiss) with compensating-ocular 12.]

PLATE 2.

FIG. 1.—Microgametocyte associating with macrogametocyte (lying free in gut). Karyosome of microgametocyte has partially broken up to form a chromidial net.

Fig. 2.—In this figure the chromidial net is seen distributed throughout the whole of the microgametocyte.

FIG. 3.—The chromidial net of the microgametocyte has become gathered together into a dumbbell-shaped network.

FIG. 4.—Stage in formation of four nuclei. The developing nuclei are still connected with the chromidial net.

FIG. 5.—The four nuclei are showing signs of becoming separated as star-shaped masses of chromatin. Part of the network is still visible.

PLATE 3.

FIG. 1.—Association of two microgametocytes with a single macrogametocyte. A chromidial net is seen in both the former.

FIG. 2.—Association of two microgametocytes (each containing four characteristic star-shaped nuclei) with one macrogametocyte.

FIG. 3.—Two microgametocytes associating with one macrogametocyte. In one, four nearly ripe microgametes are seen; in the other, a dumbbell-shaped network is present.

FIG. 4.—Four mature male gametes formed. A chromatic spindle is seen in the macrogamete.

FIG. 5.—Macroschizont, the nuclei in the form of networks. Numerous chromatin particles are scattered through the cytoplasm.

FIG. 6.—Group of macromerozoites with nuclei in a chromidial condition.

The Natural and Induced Resistance of Mice to the Growth of Cancer.

By E. F. Bashford, M.D., J. A. Murray, M.B., and W. Cramer, Ph.D.

(Communicated by Dr. J. Rose Bradford, F.R.S. Received December 10, 1906,—Read January 17, 1907.)

In the present paper we propose to give an account of experiments conducted during the past three years on the means whereby mice may be completely protected against the inoculation of transplantable carcinomata, which grow readily in normal mice. At the same time we shall show that these experiments throw fresh light on the nature of cancer. The Executive Committee and the Pathological Sub-Committee of the Imperial Cancer Research Fund have been informed of the progress of these investigations, and some provisional results have also been laid before the General Committee at the Annual Meetings. The experiments are being continued, but some of the results which have accumulated make it advisable to give an account of the stage at which they have now arrived. They are based mainly on a study of 23 transplantable carcinomata of the mamma of the mouse, and of other malignant new growths which could not be propagated artificially.

In these experiments we have used, for the most part, the growth of Jensen's tumour in normal animals to bring out the changes induced in protected animals and the refractoriness of insusceptible animals; but we shall also employ another tumour (XXVII) of different histology, which grows equally well under artificial propagation. We have employed Jensen's tumour as a standard for the following reasons:—(1) It was the only tumour used to control some of our earlier observations; (2) With proper precautions it gives regularly 85 to 100 per cent. of successful inoculations in a large number of animals; (3) Within ten days the inoculation of 0·01 to 0·02 gramme of tumour tissue gives large easily recognisable tumours, often 1·5 grammes in weight; (4) We have fully demonstrated that it may produce large metastases, that it may extend by the blood or lymphatic streams, and behave under experimental conditions typically as a malignant new growth; (5) Its rate of growth is not exceeded by any mouse tumour now being propagated; (6) It is in the hands of most investigators throughout the world, who will be able to repeat our observations. It therefore fulfils better than any other tumour the requirements of such a standard.

Historical.

Jensen stated in papers appearing in 1901, 1902, and 1903* that he had observed the complete disappearance of tumours from mice that had been inoculated successfully. He added, however, that the "results of attempts to cure mice of tumours have hitherto been uncertain; at times they have been positive to an unexpected extent, at other times they have been entirely negative." For this reason he postponed the detailed discussion of his experiments till he had come to a clearer understanding of their significance, and to more reliable results. Referring to the fact that 40 to 50 per cent. of the total inoculations of his tumour had been unsuccessful, Jensen interpreted this to mean that the mice were protected by some natural means. It seemed to him impossible to explain the fact that a tumour which was growing well at the time of its removal from one mouse should be unable to grow in other mice, except on the assumption that the tumour had been transferred to a more refractory group of mice; but he stated, "it appears that the nature of the inoculated tissue is of no little importance for the success attained. So far as it is possible to form an estimate, the results appear less certain when small young tumours are used which are actively growing, and the percentage of successful inoculations is higher if the healthy portions be selected from older and larger tumours." Jensen especially pointed out that mice which had been unsuccessfully inoculated at the first essay were also refractory to subsequent inoculations; but he was careful to point out that it would be a mistake to assert that the second negative result was solely the consequence of natural refractoriness, since it would be wrong to assume that the material inoculated and absorbed in the first instance had not contributed to the exemption subsequently exhibited.

Borrel† drew attention to the difficulties attending primary transplantation and recorded suggestive experiments directed to obtaining a polyvalent immune serum against human cancer, on the assumption that distinct sera might be necessary for tumours of different histological types.

In November, 1903, we were able to add the study of Jensen's tumour to that of tumours discovered by ourselves, and we at once began investigations along the lines indicated by Jensen. In our earlier experiments, the successful inoculation of Jensen's tumour fluctuated between 20 and 90 per cent. of the number of animals inoculated.

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* "Nogle Forsøg med Kraeftsvulster," Lecture, December, 1901; "Forsøg med Musecancer," Lecture, April, 1902, 'Biolog. Selskabs Forhandlinger, Köbenhavn,' 1901—02; "Experimentelle Untersuchungen über Krebs bei Mäusen," 'Centr. f. Bakt.,' vol. 34, 1903.

† "Epithélioses infectieuses et Epithéliomas," 'Annales de l'Inst. Pasteur,' vol. 11, 1903; 'Roy. Soc. Proc.,' vol. 73, 1904.

In a series of papers* we have drawn attention to the greater susceptibility of young than old mice, to the important influence on the success of transplantation, of the character and phase of the tumours inoculated, the dosage, and site of inoculation. In the course of a careful study of the process of absorption of tumours after exposure to radium, we showed its identity with localised changes in the majority of propagated tumours, changes which were found to have become general in the only case we had then noted where a tumour of three weeks' growth and of 1 cm. diameter had actually diminished in size.† While recognising the importance of hæmorrhage as an integral part of the process of absorption both when spontaneous and following exposure to radium, we pointed out that the phase of growth of the tumour was also of great and probably decisive importance. These observations harmonised well with Jensen's experience, and tended to explain the irregularities in the disappearance of tumours under his observation. Our position was summarised as follows:—"No conclusion has yet been arrived at as to what are the essential and what the subsidiary features in this prolonged proliferation. The variations make it difficult to correlate different observations, and to be sure of their proper interpretation. Efforts to influence growth by immune (cytolytic) serum and other means have, in consequence, not yet led to any definite conclusion."

Clowes,‡ working also with Jensen's tumour, obtained 30 per cent. of success in normal animals. He recorded spontaneous absorption in from 15—20 per cent. of his transplanted tumours. The animals in which spontaneous absorption had occurred were stated to possess a specific antibody in their serum, which, on injection into animals with transplanted tumours, exerted a curative effect. In only one case out of 20 mice so treated had the beneficial influence of the immune serum been missed. In view of the low percentage of success obtained, the slow development of the tumours, and Jensen's and our own experience, we then expressed scepticism of the curative results claimed by Clowes. In his later papers these results are quoted, but a repetition of the positive results has not been recorded. Evidence is adduced to show that the animals which have recovered spontaneously cannot be successfully inoculated. This is regarded as an action of anti-bodies. The presence of anti-bodies is further supported by experiments in which tumour material had been exposed, in the test-tube, to the action of the serum of spontaneously cured mice. On inoculation, such material gave 12 per cent. of success, while material similarly treated with normal mouse serum gave 31 per cent. These three sets of observations are progressively evidence of a more limited action of the reputed immune sera. The age of the animals used is not stated. In March, 1906, Ehrlich* remarks with reference to these experiments: "So berichtet Clowes, dass Mäuse, bei denen Tumoren spontan zur Resorption gelangen, in ihrem Serum carcinomfeindliche Stoffe enthalten. Allerdings lassen sich gegen die Deutung dieser Versuche mancherlei Einwendungen machen." Other papers, in which these and other observations are used as evidence of the infective nature of cancer, have also been published by Clowes, Gaylord, and Baeslack, from the Buffalo Cancer Laboratory, U.S.A.†

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* First and Second Scientific Reports of Imperial Cancer Research Fund, 1904, 1905; 'Roy. Soc. Proc.,' B, vol. 78, 1906.

† We recorded the precautions we had taken in searching for tumours undergoing spontaneous absorption. Clowes has taken this criticism of our own procedure as implying that in our opinion his "spontaneous recoveries were ulcerations" ('Brit. Med. Journ.,' December 1, 1906). Regression of inflammatory swellings may take place without ulceration, and our remarks apply to our own doubtful "tumours" of less than 14 days' growth, within which time Clowes made no mention of tumours having appeared.

‡ "Preliminary Communication regarding an Immune Body Capable of Inhibiting the Development of Cancer in Mice," 'Johns Hopkins Hosp. Bull.,' April, 1905; "Further Evidence of Immunity against Cancer in Mice after Spontaneous Recovery," Clowes and Baeslack, 'Medical News,' November 18, 1905.

Michaelis‡ obtained success in the same proportion as in normal animals, on inoculating mice previously treated with tumour material killed by chloroform and other chemical agents.

Ehrlich§ found that from 60 to 90 per cent. of mice inoculated unsuccessfully with spontaneous (especially hæmorrhagic) tumours were refractory on subsequent inoculation with a tumour giving 75 to 100 per cent. of success in normal animals. Further, animals unsuccessfully inoculated with a tumour giving nearly maximal percentage of success in normal mice, were highly refractory to subsequent inoculation. This resistance was manifest whether the second inoculations were made with the same or other tumours (pan-immunity). Animals already successfully inoculated with a quickly growing tumour could not again be inoculated with a rapidly growing tumour

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* "Experimentelle Carcinomstudien an Mäusen," 'Arbeiten a. d. Kgl. Inst. f. exp. Therapie,' 1906.

† "On Spontaneous Cure of Cancer," 'Surgery, Gynæcology, and Obstetrics,' June, 1906; "Incubation of Mouse Tumours," 'Journ. of Exp. Medicine,' August, 1906; "A Study of the Influence Exerted by a Variety of Physical and Chemical Forces on the Virulence of Cancer in Mice," 'Brit. Med. Journ.,' December 1, 1906; "Evidences that Infected Cages are the Source of Spontaneous Cancer developing among Small Caged Animals," *ibid.*

‡ 'Zeitschr. f. Krebsforschung,' vol. 4, 1906.

§ "Experimentelle Carcinomstudien an Mäusen," 'Zeitschr. f. ärzl. Fortbildung,' 1906, and *loc. cit.*

(atreptic immunity): the tumour already established withdraws special nutritive substances (substance X) from the circulation, so that the new graft cannot grow. A similar explanation is invoked for the rarity, or small size, of metastasis in mice with large transplanted growths. Repeated inoculation of negative mice leads to a very refractory condition in the animals which never develop tumours.

Rats can be successfully inoculated with rapidly growing mouse tumours. Growth proceeds for a time and then ceases (atrepsie), and the animals are then refractory to further inoculation (active immunity).

Summary of the Present Investigation.

After discussing:—

1. The distinction to be drawn between the conditions of origin of a sporadic tumour and the conditions of growth of cancer in mice, and its bearing on experimental investigations.

2. The importance of natural variations in the resistance of mice to inoculation and of inherent variations in the energy of growth of the tumour cells.

We shall adduce evidence that:—

3. Mice in which a growing carcinoma has been spontaneously absorbed may be completely protected against subsequent inoculation of the same growth, and to a lesser extent against other and different growths. Similarly, protection follows absorption of tumours after exposure to radium.

4. Protection may be induced, or, when naturally present, enhanced by the inoculation of tumour material when no growth has resulted. This protection is specific, and has only been induced in mice by the inoculation of mouse tumours, and not by those of strange species. This specificity has an important bearing on the nature of cancer.

5. Protection can also be induced by the inoculation of normal mouse tissues, and particularly by the inoculation of blood.

We shall discuss the probable nature of the protection conferred; its action by means of the body fluids, at any rate in part, leading to the death of the cancer cells after short sojourn in the body of protected animals.

(1) THE DISTINCTION TO BE DRAWN BETWEEN THE CONDITIONS OF ORIGIN OF A SPORADIC TUMOUR AND THE CONDITIONS OF GROWTH OF CANCER IN MICE, AND ITS BEARING ON EXPERIMENTAL INVESTIGATIONS.

When the energy of growth of the cells of a propagable tumour is great and a high percentage of all inoculations are successful, the variations in the natural resistance of the mice become of subsidiary importance. On the contrary, they are of great practical importance when the energy of growth is low, and in the primary transplantations of sporadic tumours. As we have shown, the growth of a sporadic tumour is probably to be regarded as heterogeneous. Growth is proceeding rapidly in one part, and slowly or actually ceasing in another. When a sporadic tumour is transplanted into a large number of mice, according to our procedure, it is subdivided into minute fragments, and not made into a general emulsion. Each fragment is separately emulsified before transplantation, and the result of transplantation is that the tumour is distributed over a large number of animals. Success, as a rule, follows only in a small proportion of the inoculations, and it appears probable that it is determined by the segregation of groups of cells of higher energy of growth in mice whose resistance to inoculation is below the average. The energy of growth of the tumour cells and the resistance of the mice together contribute to the positive result. In this way the cells most likely to continue to grow are separated and, by the repetition of the process, a tumour is propagated by transplantation from one series of mice to another. We may illustrate this by a summary of the transplantation of some sporadic tumours.

We have found that malignant new growths are encountered in mice of all ages taken at random in a proportion of one tumour in 3500 animals. The final results of transplanting 32 spontaneous tumours of the mamma show that 2278 of the mice inoculated survived a sufficient time* to permit of a final estimation, and 72 tumours have developed, *i.e.*, one inoculation in 31·1 is successful, or 3·2 per cent. The conditions for successful transplantation are at least 100 times more frequent in mice than spontaneous tumours.

Fifteen out of 32 sporadic tumours transplanted gave negative results on 1073 inoculations. Many of these tumours were apparently very rapid in their rate of growth in the animal spontaneously affected, and extensive metastasis was present in the lungs after death.

Ehrlich records similar results on primary transplantation of 94 spontaneous tumours: 1504 inoculations were made and 41 tumours were obtained in the inoculated animals (2·8 per cent.). The percentage of success (8·2 per cent.) of the primary transplantation of the tumours (14 in number) which gave positive results at all, was much higher than in our experiments. Apparently our method of primary transplantation by small grafts in a large number of young animals, gives success with tumours which would be completely negative when propagated in the way he has adopted (large doses and smaller number of animals).

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* The results of the primary transplantation of sporadic tumours have been calculated on the number of mice surviving three weeks later.

The following experiments were undertaken because of the difficulties met with in obtaining a positive result in the primary transplantations of spontaneous tumours. It was of importance to determine whether the spontaneously affected animals were more suitable for transplantation, both of their own tumours (on the analogy of metastasis) and of other spontaneous tumours. The difficulty of transplanting many mouse tumours, especially those with extensive hæmorrhage, has led observers who had failed to transplant them to conclude that those tumours were of low "virulence." We had already succeeded* in three instances where others had failed in transplanting these hæmorrhagic tumours, the daughter tumours being also hæmorrhagic, and had observed that they produced extensive metastasis in the animals primarily affected. We were, therefore, of the opinion that it was wrong to conclude from the difficulty of transplantation that they were of low virulence.

We have prolonged the lives of mice presenting spontaneous tumours by removing the primary growth by operation, wholly or partially. This has permitted us to study their suitability for transplantation, both for their own and for other spontaneous tumours, and to compare it with the suitability of normal animals.

The opportunities for such experiments are rare. Up to the present 13 animals presenting spontaneous tumours have been inoculated with their own and other spontaneous tumours. The results for 11 of these animals are now available, and are as follows:—

Two mice (XXXIII and XXXIV) could be successfully inoculated with their own tumours, and in the case of one of them (XXXIII) three normal animals also developed tumours out of 97 inoculated and surviving three weeks after inoculation. In the case of the second mouse, her tumour gave negative results in 140 normal animals, although successfully inoculated into herself.

A third spontaneous tumour (XXXII) gave four tumours in 156 normal animals, was negative in the mouse spontaneously affected, but positive in another mouse (XXXIII) with spontaneous cancer. A fourth spontaneous tumour (XL) inoculated into 49 normal animals, failed to grow and was likewise negative in two mice with spontaneous cancer (XXXVII and XXXVIII). A fifth spontaneous tumour (XXX) gave one tumour in 72 inoculations into normal animals, but failed to grow in three other mice (XXV, XXVII and XXVIII) spontaneously suffering from cancer, as well as in the mouse herself. Three inoculations were made in each of these four mice.

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* Second Scientific Report, 1905, Part II, pp. 19 and 30.

A mouse which had been repeatedly inoculated unsuccessfully with Jensen's tumour developed a spontaneous growth of different histological type (XXXVIII) seven months later. This tumour, transplanted into 86 normal animals, gave rise to 20 tumours, of which 18, after attaining a diameter of 5 mm. and over, disappeared spontaneously. The mouse in which this tumour had developed remained protected against Jensen's and refractory to four spontaneous tumours.

The experiments are very few in number, and only tentative conclusions can at present be drawn from them.

Five spontaneous tumours were transplanted into 11 mice in all, spontaneously affected with cancer. The results above recorded show that transplantation does not reveal conditions exceptionally favourable for growth in them, as compared with normal animals. The animal itself is only exceptionally successfully inoculated.

Bearing in mind the small number of spontaneously affected animals inoculated as compared with the large numbers of normal animals, these experiments show that spontaneously affected animals are not greatly more susceptible to cancerous inoculation than normal animals.* Tumours which do not grow in normal animals rarely grow when transferred to other parts of the animal's own body, and not at all in other spontaneously affected animals.

These facts, together with the development of a tumour in a mouse previously inoculated unsuccessfully with Jensen's tumour, further emphasise the distinction to be drawn between the conditions of origin and the conditions of growth. They also seem to render superfluous any subsidiary assumption of a constitutional condition as necessary in addition to the primary cancerous transformation in the development of spontaneous cancer.†

* The results of the above experiments might, on superficial examination, appear to justify the opposite conclusion to that drawn by us. The tumours of mice XXXIII and XXXIV were transplanted successfully in those mice themselves in single experiments, as against only three successes out of 99 attempts in normal animals in the one case, and with negative results in 176 attempts in the other. Of all the sporadic tumours transplanted into other mice suffering naturally from cancer, XXXII alone grew in a single experiment, giving at the same time only four successes out of 156 attempts in normal mice. It would thus appear that 100 per cent. of success had been attained on three separate occasions when transplantation was effected into mice naturally suffering from cancer, as against very low percentages in normal mice. The results speak for a greater suitability for inoculation on the part of individual mice, but not for a greater suitability on the part of all mice which are naturally the victims of cancer. The conclusion as to individual susceptibility is only justified if the distinction be borne in mind between transplanting a tumour into the mouse in which it grew primarily, and into any mouse afflicted with spontaneous cancer. The two cases are not the same, as pointed out later on. The fact that a mouse is suffering naturally from cancer does not imply a greater suitability for transplantation generally.—*Added Feb.* 8, 1907.

† *Cf.*, however, p. 184, Summary.

A clear conception is required of the necessity for interpreting the results of the experiments about to be described as bearing directly on the conditions of growth only and their artificial modification. By inference, they have also important bearings on the nature of cancer; but we desire to enforce caution in anticipating prophylactic or therapeutic actions as likely to follow the use of those measures we have found efficacious in raising the resistance of animals to transplantation.

(2) THE IMPORTANCE OF NATURAL VARIATIONS IN THE RESISTANCE OF MICE TO INOCULATION, AND OF INHERENT VARIATIONS IN THE ENERGY OF GROWTH OF THE TUMOUR CELLS.

In previous communications we have directed attention to variations in the susceptibility of individual mice to inoculation with a number of different mammary tumours of the mouse. The natural insusceptibility to inoculation to which we particularly wish to draw attention in the present communication is that which we have stated was still present after the disturbing influences of race, age, dosage, and fluctuations in the powers of growth of the cancerous cells, have been allowed for in artificial propagation of transplantable tumours. In earlier papers we used the natural resistance of the animals as an indicator of the variations of the power of proliferation of the cancer cells. We shall now conversely use the tumour-cells as indicators of the susceptibility of the inoculated animals. In such experiments the number of animals necessary is inversely proportionate to the percentage of successful inoculations attainable. Where the tumour gives a maximal success in normal animals, the number necessary in each experiment can be reduced, and must be correspondingly increased when the percentage of success in normal animals is lower.

Experiments in which less than 60 per cent. of the normal animals develop tumours are open to grave objections, and will not be employed in this paper.

Until we can experimentally determine the development of cancer *de novo*, we are necessarily confined to a study of its growth and the conditions which favour or hinder it when cancerous tissue is inoculated into mice. The differences between mice of different susceptibility are expressed *quantitatively* in differences in the percentages of successful inoculations.

In all these experiments we have to take account of two variable factors of prime importance which are beyond experimental control:—

(1) The varying natural susceptibility of the mice;

(2) The varying energy of growth of the tumour cells.

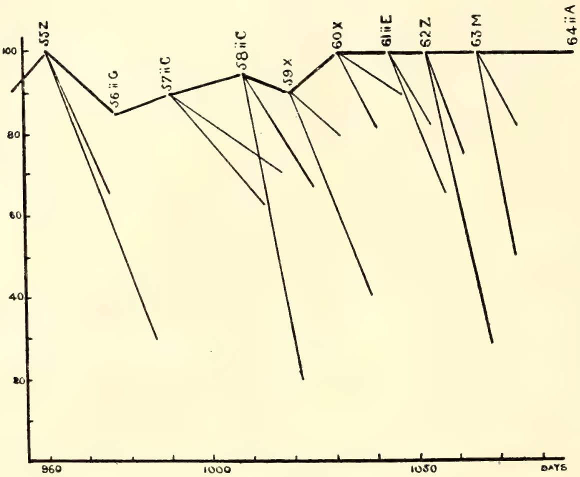
These vary independently of each other, and under normal circumstances, either alone or in conjunction with one another, they determine the phenomena of growth under artificial propagation. Hence, extensive control experiments are necessary at every step in attempts to modify growth artificially, for a low percentage of success may be due to a refractory condition in the mice used, or to a lower energy of growth of the tumour cells. In addition, other factors of less importance can introduce variations which are detailed below, and must be excluded.

The following precautions have been taken to obtain results which may be legitimately compared. The differences which have been elicited in these experiments are in many cases slight, and dependent on very subtle alterations in the animals. In order to measure them, it has been found advisable to work on the margin of maximum success in transplantation. In order to obtain comparable results under otherwise similar experimental conditions, attention must be paid to (1) the dose of tumour material, (2) the age of the animals, and (3) the site of inoculation. The inoculations must be carried out in such a way that "tumour formation" or "no tumour formation" can be determined by the conditions of the animals for the most part. We have worked mostly with a "minimal tumour-forming" dose, although multiples of this dose have been employed for special purposes. Large doses frequently lead to the development of tumours in animals which are completely protected against, or refractory to, smaller doses. Then the refractory condition, or relative protection, may only be indicated by the smaller size and less rapid growth of the resulting tumours, as compared with normal mice. A cleaner result is obtained when the dose, while sufficient to produce tumours in normal animals within 10 days, fails to lead to any proliferation whatsoever, thereby indicating the presence of abnormal refractoriness in the animals whose resistance is being tested. Our animals not being very highly protected, many of the differences we shall record are only clearly elicited by the use of small doses. The use of large doses obscures them when results are recorded as percentages of successful inoculations. The difference between different sites is best brought out by small doses, and obscured by the use of large doses.

Young mice are more susceptible to inoculation than old animals. The difference in percentage of success may be as great as 60 per cent. between animals of seven weeks, on the one hand, and those of one year old, on the other hand. In the following experiments the control animals have always been chosen of the same size (and therefore of approximately the same age) as the animals whose resistance is tested. This precaution is necessary in all such experiments, because the duration of the preliminary treatment whose effect is under discussion, of itself entails an alteration in the susceptibility of mice whose total duration of life is short.

During the continued propagation of Jensen's tumour in the last three years, series of inoculations have been obtained repeatedly with maximal percentage of success. The method of repeated subdivision of the parenchyma into the small grafts necessary for the analysis of growth, showed that each such maximum was followed by a rapid and great diminution in the percentage of success, on which again an increase ensued, till a fresh maximum was encountered. The complication which these fluctuations introduce has been in great part avoided in the following way.

By choosing a suitable interval for inoculation of tumours selected from series with from 90 to 100 per cent. of success, and especially by increasing the initial dose introduced into each animal from 0·01 to 0·05 gramme, we have been able to evade the diminution usually following each maximum for a considerable number of transferences. This result is artificial. It does not indicate an increased "virulence" of the tumour cells, or a continuous growth of uniform energy on their part. It is in complete harmony with the results of the experimental analysis of the growth of cancer as we have already described it.



Graphic Record of Propagation through ten Passages of a single Strain of Jensen's Carcinoma in which success has not fallen below 85 per cent.

In the case of other tumours we have obtained the same result in the same way, but most speedily in the case of spontaneous tumour XXVII, which at the third transplantation gave 80 per cent. of successful inoculations, and at the fifth 98 per cent., the success of the primary transplantation being only 20 per cent.

Separate strains of Jensen's tumour have been propagated, as indicated in the accompanying graphic record, through successive transplantations, without the percentage of success in the direct line of propagation having fallen below 85 per cent. This is merely the result of an artificial selection of the tumours obtained at each successive transplantation. It furnishes a special case of the process already described in detail, and receives mention here because of the great convenience of maximum success in inoculation for controlling the experiments with which the present paper deals, and because other observers have not yet succeeded in attaining to this maximum of transplantability in the case of Jensen's tumour. These results, together with the other facts mentioned on a previous page, entirely do away with the statements that Jensen's tumour is not virulent. We have sent our strain of Jensen's tumour to Professor Uhlenhuth and Professor von Dungern. The primary results they have communicated to us show that, in English mice, Professor Uhlenhuth obtained 90 per cent. of success and Professor von Dungern 85 per cent. In German mice the results were 90 per cent. and 50 per cent. respectively.

The slight differences which suffice to determine the success or failure of transplantation are exemplified in the following series of experiments, which were devised to demonstrate the inherent fluctuations of nearly allied strains of the same tumour under the same conditions at each transference. Incidentally the experiments revealed the danger of comparing together inoculations not strictly in the same site.

Two strains of the same tumour were propagated simultaneously on opposite sides of the body of the same mice. The strains used were chosen because of moderate powers of growth, and the fluctuations they had exhibited. On a series of 11 passages, it was found that the axilla was invariably a more suitable site than the dorsal subcutaneous tissue: 59 mice out of 286 developed tumours both back and front, 18 mice had tumours only on the back, and 59 only on the front. The difference between the dorsal and ventral sites is in all probability comparable to that already recorded by us between the dorsal subcutaneous tissue and the peritoneal cavity. The most natural explanation of the difference would seem to be that the connective tissue reaction, which we have shown is specific and without which the grafts cannot grow, is more readily supplied by the connective tissue of the mammary region.

In these experiments we also noted that whenever a tumour of the low percentage strain developed on the back, it almost always had its fellow in the axilla, the converse not holding good.

When the greater suitability of the axilla had been established beyond all possibility of doubt, the experiment was continued through five successive passages in the right and left axilla. The results, as a whole, are more uniform in this series. Out of 95 mice inoculated, 59 developed tumours on both sides, five on the right side only, and 11 on the left side only. The two strains, propagated in the right and left axilla respectively, fluctuated practically independently of one another, at one time a higher percentage was obtained in the right axilla, at another time in the left. It is to be noted, however, that whenever the strain with the lower percentage for the time being was able to establish itself on its side, the tumour giving a higher percentage always succeeded in establishing itself on the opposite side of that individual mouse, so that a tumour of the strain with lower percentage never occurred singly, each had its fellow on the opposite side in the more numerous tumours of the high percentage strain. Hence the differences in percentage of success between the two strains are considered to be due to fluctuations in the powers of proliferation of the cells of each, and to be independent of the susceptibility or resistance of the mice in which they are, or have been, growing. If the resistance of the mice in the right or left axilla be assumed to be similar, then this resistance really serves merely as a measure of the varying qualities of the tumour cells. It plays the part of a sieve, as it were, which keeps back the cells with less powers of assimilation and growth, and permits those others to pass which can survive the hindrance to their continued existence and multiplication.

(3) MICE IN WHICH A GROWING CARCINOMA HAS BEEN SPONTANEOUSLY ABSORBED MAY BE COMPLETELY PROTECTED AGAINST SUBSEQUENT INOCULATION OF THE SAME GROWTH AND TO A LESSER EXTENT AGAINST OTHER AND DIFFERENT GROWTHS.

Animals in which tumours have developed and then have been absorbed are highly refractory to further inoculation. This protection may be absolute. This fact is sufficiently evidenced by the following experiments. The animals were re-inoculated in another part of the body after complete disappearance of the first tumour. The control animals were inoculated in the same place as the second experiment of the spontaneously-recovered mice.

PROTOCOL TO ILLUSTRATE ABSOLUTE PROTECTION AFTER SPONTANEOUS ABSORPTION.

A. Experiment 61, ii M (1). Tumour taken from a mouse of transplantation 60, Series V, was transplanted into the right axilla of 20 normal mice. Eight tumours developed in the 12 animals surviving after 10 days (64 per cent.).

(2) The same tumour was also inoculated at the same time into 36 mice which had been previously inoculated successfully in the right axilla, but in whom the tumours evident after 10 days ultimately disappeared. Two small tumours developed in the 33 animals surviving after 10 days (6 per cent.).

B. (1) Experiment 62, ii K. Thirteen days after the inoculation recorded above, 15 of those mice which had presented no tumours were re-inoculated in the left axilla, with tumour of transplantation 61, Series ii N. No tumours developed, while 15 normal animals inoculated with the same tumour presented 13 tumours after 10 days (87 per cent.).

(2) Experiment 62, ii L. The remaining 15 mice of Experiment 61, ii M, were inoculated in the left axilla on the same day with a tumour of transplantation 61, Series ii J. No tumours developed, while 15 normal animals inoculated with the same tumour all presented tumours after 10 days (100 per cent.).

The protected animals were further inoculated unsuccessfully, and were then used to test the properties of the blood.

Protection similar to that afforded against Jensen's tumour by its spontaneous absorption is also manifested by animals in which other spontaneous tumours of different histology have grown for a time, and the tumours been absorbed. Of the tumours on which such observations have been made, XIX was an adeno-carcinoma with cystic spaces and widespread hæmorrhages; XXVII another adeno-carcinoma tending to become alveolar, *i.e.*, approaching the histological type of Jensen's tumour, and XXXVII a malignant adenoma with little tendency to form solid alveoli, while XXXVIII was also an adeno-carcinoma with small acini interspersed with solid alveolar portions.

The animals in which tumours have disappeared after exposure to radium are refractory in the same way and to a similar degree as those in which absorption has occurred spontaneously. This is only what was to be expected in view of the probable identity between the process of absorption in the two cases, as described in a previous paper.

The general conclusion can be drawn that recovery from one form of tumour of the mamma protects against subsequent inoculation with other forms. When protection is absolute in the case of the tumour recovered from, it is not necessarily also absolute for other tumours. There is thus a suggestion of a specificity in the protection. The mice which have recovered from experimental cancer have undergone a change conferring on them a degree of protection as high as that presented by the most refractory of normal animals. This alteration is not confined to the tissues in the immediate neighbourhood of the spontaneously absorbed tumour; it must have become general by means of the body fluids.

(4) PROTECTION MAY BE INDUCED OR, WHEN NATURALLY PRESENT, ENHANCED BY THE INOCULATION OF TUMOUR MATERIAL WHEN NO GROWTH RESULTS.

We have already pointed out that the animals which have been unsuccessfully inoculated with small fragments of a tumour giving a low or moderate percentage of success may, on re-inoculation, give a positive result in a proportion little inferior to a series of control animals. The negative result in the first instance was thus due not entirely to the inoculation of refractory animals, but largely to the low powers of proliferation of the tumour-cells introduced.

PROTOCOL.

Tumour XV. Mouse with a large tumour of the fifth transplantation received from Cologne (Dr. O. Schmidt), together with 10 normal mice.

We effected the sixth transplantation into 10 normal Cologne mice, and 147 normal English mice. In the English mice, three tumours presented themselves in the 61 mice which survived 10 days after inoculation (5 per cent.). Four tumours developed in the 10 Cologne mice, all having survived (40 per cent.).

One month later one of the tumours which had developed in a Cologne mouse was transplanted into 45 English mice and the three Cologne mice which alone had survived out of the six negative animals in the previous experiment. All the three Cologne mice developed tumours, 100 per cent., while only two tumours developed in the 20 English mice surviving after 10 days.

Repeated re-inoculation of the negative animals with small doses of tumour leads to the sifting out of a residuum which may possess a high degree of resistance. As the end result of such a process of elimination by successive inoculation, animals can be obtained in which, while similar control animals give 70 to 80 per cent. of success, only from 10 to 12 per cent. of successful inoculations are obtained. When, however, the small number of animals unsuccessfully inoculated in an experiment with sub-maximal success are re-inoculated, they evince a decided increased refractoriness as compared with normal animals. Some change in the animals following upon the absorption of inoculation material is not an unimportant factor in the development of this condition, as already suggested by Jensen. That such is the case seems more than probable from the result following, when the initial and subsequent inoculations are made with large doses, more than 0·05 gramme of tumour material. Here the refractory condition is increased in a much shorter series of inoculations, so that already at the third or even at the second inoculation, a diminution of 50 to 60 per cent. may be observed as compared with normal animals. This result confirms Ehrlich's observations on the same subject.

PROTOCOL TO ILLUSTRATE INCREASED RESISTANCE AFTER A PRECEDING NEGATIVE INOCULATION.

Nineteen normal mice were inoculated in the left axilla with 0·04 gramme of a tumour of transplantation 62, Series ii C,* the experiment being labelled "63 S." All the mice survived, but two only presented tumours after 10 days. Thirteen days after this inoculation, 13 of those 17 negative animals were re-inoculated in the right axilla with a tumour of transplantation 63, Series M, the experiment being labelled "64 W." Of the six animals surviving after 10 days, only one presented a tumour (17 per cent.). Of 15 normal mice which had been similarly inoculated among the 11 surviving after 10 days, eight presented tumours (72 per cent.).

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* Transplantation 62, ii C, had given 19 tumours in 19 mice (100 per cent.). The low percentage obtained in 63 S, and the negative result in 63 T, the experiments providing

Twenty-three normal mice were inoculated in the left axilla with 0·04 gramme of another tumour of transplantation 62, Series ii C, the experiment being labelled "63 T." No tumours developed in the 20 animals surviving after 10 days. Thirteen days after the preceding inoculation, 19 of the mice were re-inoculated in the right axilla with 0·03 gramme of a second tumour of transplantation 63, Series M, the experiment being labelled "64 V." Of the 18 mice surviving after 10 days, five presented tumours (27 per cent.). Fifteen normal mice were similarly inoculated, 12 survived, and 10 presented tumours after 10 days (81 per cent.).

Differences in the size of dose of tumour probably explain the apparently contradictory experience of others who have failed to produce protection in this way, and of our own earlier experiments where the re-inoculation of the negative animals gave a percentage almost as high as in the first instance. The mass of tumour absorbed in the course of three or four negative inoculations with 0·05 gramme is so great that the animals, although never developing tumours, cannot be regarded as comparable with animals in our earlier experiments which, inoculated the same number of times, have only received relatively insignificant doses (0·01 to 0·02 gramme).

Refractoriness to various spontaneous mouse tumours is apparently reciprocal. Animals which have been unsuccessfully inoculated with large doses of one spontaneous tumour, whether carcinoma or sarcoma, are less suitable for the transplantation of other carcinomata than are normal animals. This fact receives a natural explanation in the results of experiments detailed below on the protection conferred by the injection of normal mouse blood. On the other hand, the inoculation of mice with tumour material from strange species—rat, cat, dog, man—has not induced a refractory state. The protection induced in mice is therefore to be interpreted, not as conferred by properties common to the malignant new growths of different species, but only of the same species, by the absorption of substances common to the tumours and tissues of the mouse.

(5) PROTECTION CAN ALSO BE INDUCED BY THE INOCULATION OF NORMAL TISSUES, AND PARTICULARLY BY THE INOCULATION OF BLOOD.

Early in 1904 we were enabled, through the courtesy of Professor C. J. Martin, F.R.S., of the Lister Institute, to study the effects of immunising

the negative mice referred to, is a phenomenon to which we have previously drawn attention (*vide* 'Roy. Soc. Proc.,' June, 1906).

The tumour tissue inoculated was in what we have designated, for convenience, the negative phase of growth.

animals, including rabbits, guinea-pigs, rats, and mice against mouse tissues (liver, kidney, testis) and tumour, all of which had been freed from blood by washing out the circulation with normal saline, and ground to a fine powder at the temperature of liquid air. The results were unsatisfactory, apart from the exhibition of a distinct specific hæmolytic action on the erythrocytes of mice on the part of the serum of animals immunised against each of the blood-free tissues and a precipitin reaction with mouse serum. In particular, no satisfactory evidence of a specific action on the living cells of mouse tumours could be obtained either in the test-tube or in the body.

We were led to investigate more fully the effects of the absorption of mouse blood on normal mice, not only because of the difficulty of working with the other tissues, but because of the following considerations: Hæmorrhage is frequent in the process of absorption of tumours after exposure to radium, and during spontaneous absorption. A number of hæmorrhagic tumours successfully transplanted in the first instance were found to be peculiarly liable to spontaneous absorption, and in their case the condition was comparable to what is now well known to occur in human chorion-epithelioma. In this connection, we are indebted to Dr. J. H. Teacher for drawing our attention, early in 1905, to the frequency of the spontaneous disappearance of the metastatic growths of chorion-epithelioma and their hæmorrhagic character. It was found that an injection of 0·3 to 0·5 c.c. of defibrinated normal mouse blood induced a definite refractory condition, even in young animals. It is not so marked four days after injection as at 10 days, and persists for at least three weeks. The repetition of the blood injection after 10 days does not markedly increase the refractory condition. The animals thus protected do not ultimately develop tumours when observed during a period of three months.

PROTOCOL TO ILLUSTRATE PROTECTION CONFERRED BY A PRECEDING INJECTION OF NORMAL MOUSE BLOOD.

Experiment 61 T. Sixteen mice received an injection of 0·3 c.c. of normal defibrinated mouse blood on the back. Five days later they were inoculated in the right axilla with 0·01 to 0·02 gramme of a tumour of transplantation 60, Series ii P. All the mice survived, and seven presented tumours after 10 days (44 per cent.). At the same time 30 normal mice were inoculated with the same doses of the same tumour, also in the right axilla. Of the 26 animals surviving after 10 days, 19 presented tumours (73 per cent.). The tumours in normal animals were throughout larger than those in the mice previously treated with blood.

Experiment 62 Q. Twenty-eight mice received 0·3 c.c. normal defibrinated mouse blood on the back. Eleven days later they were inoculated in the right axilla with 0·01 to 0·02 gramme of a tumour of transplantation 61, Series S. Three tumours developed in the 15 animals surviving after 10 days (20 per cent.). At the same time 30 normal animals were inoculated in the same place with the same dose of the same tumour. All survived, and 19 presented tumours after 10 days (66 per cent.). The tumours in treated animals were smaller and grew less rapidily than those in the normal mice.

Experiment 62 Z. Fifteen mice received an injection of normal defibrinated mouse blood. Ten days later they were inoculated in the right axilla with 0·02 gramme of a tumour of transplantation 61, Series ii E. All the mice survived, and 10 presented tumours after 10 days (67 per cent.). At the same time 15 normal animals were inoculated in right axilla in the same way. Ten mice survived, and all presented tumours after 10 days (100 per cent.). The same relation was again observed between the tumours in the two series.

Experiment 61, ii E. Thirty-nine mice received 0·3 c.c. of normal defibrinated mouse blood on the back. Seven days later they were inoculated in the right axilla with 0·02 gramme of a tumour of transplantation 60, Series X. Of the 38 mice surviving after 10 days, 15 presented tumours (39 per cent.). At the same time 20 normal animals were similarly inoculated. All the 15 animals surviving after 10 days presented tumours (100 per cent.). The same difference in the size and rate of growth was observed in the animals treated with blood.

The difficulty of readily obtaining large numbers of young mice artificially protected against carcinoma has been a hindrance to the studies of this kind. We have depended on the selection of animals which had been negative to repeated inoculations, or on collecting mice in which spontaneous absorption had occurred. Both proceedings take up a large amount of time and lead to experiments being conducted under unfavourable conditions, owing to the greater suitability of young animals for growth (and therefore for control experiments). By means of a preceding injection of blood, however, the initial stages of the refractory state can be quickly induced in young mice, and the condition then enhanced by suitable doses of tumour material. When once the initial stages have been passed, there is little difficulty in increasing the refractoriness.

Experiments conducted in this way appear to show that normal mouse serum (0·3 to 0·5 c.c.) free from corpuscles does not confer protection to anything like the same extent, if at all. The blood-corpuscles, suspended in saline after being freed from the serum by centrifuging, on the contrary, were nearly as efficacious as full defibrinated blood. The blood was injected into the dorsal subcutaneous tissue over the root of the tail. The inoculations were then made into the axilla, either on one or both sides. The absence of the same degree of protective action after the injection of serum appears to show that it is not due to mere super-addition of protective substances normally present in the blood of the mouse, but rather that the protection results from the active intervention of the living tissues in dealing with the injected corpuscles.

Although young mice are much more susceptible to inoculation, no difference could be discerned between animals treated with the blood of young and old mice respectively.

The blood of other nearly allied animals (rat, rabbit, guinea-pig) called forth no such alteration, so that the action is strictly specific to the mouse.

These effects are best brought out when the inoculations are made with the smallest convenient dose capable of giving rise to maximal percentages of tumours and which lies in our experiments between 1 and 2 centigrammes. When larger doses (5 centigrammes) are employed, the percentage of tumours developing in the treated animals rose, although it did not reach that in control animals, and the tumours which did develop also remained smaller. In one experiment, however, in which massive doses were inoculated, tumours subsequently developed in the animals in which no growth had appeared within 10 days. Here, apparently, the larger dose of tumour had exhausted the protective property, and subsequently growth was possible. The resistance of animals in which spontaneous absorption has occurred has not been overcome in the same way by increasing the dose of tissue inoculated. Whether this points to a difference in degree only, or a qualitative difference in the protection in the two cases, remains to be determined.

The nature of the action which the body fluids of protected animals have upon tumour tissue in the days immediately following transplantation has been approached in the following manner, after failure to obtain satisfactory results in the test-tube. The failure signifies nothing particular, and may be due either to low degree of protection or imply merely that a complex action takes place in the body, and one we have not reproduced *in vitro.*

A number of animals in which tumours had spontaneously disappeared were inoculated with large doses of tumour tissue, and at the same time twice as many normal animals were inoculated in the same way. After varying intervals the implanted tissue was removed from the protected animals and transferred to young normal animals, and the results compared with those obtained by re-inoculating the tissue removed from a similar number of the control animals, sufficient of whom were left alive to prove the vitality of the tissues inoculated in the first instance. These experiments are still in progress, but there seems little doubt that, as compared with normal animals, the tumour tissue rapidly dies in those protected by spontaneous absorption.

Summary.

Many apparently contradictory statements as to induced resistance of mice to inoculation may be harmonised when due attention is paid to (1) the susceptibility of young mice and the refractoriness of old mice, (2) to the variations in success which depend on the size of the dose of tumour inoculated, and (3) on the varying qualities of the cells of any one tumour

at different times according as they are in the positive or negative phases of growth.

When small doses of tumour, 0·01 to 0·02 gramme, are unsuccessfully inoculated into young and susceptible mice, a second inoculation is often successful; similar doses will give negative results in a large proportion of adult animals at the first inoculation, and also on subsequent inoculation. The differences between young and old mice and between the employment of small and large doses explain contradictory statements to the effect that apparently refractory animals of a primary inoculation can or cannot be subsequently inoculated. When large doses of growing tumour are successfully inoculated into young mice, giving nearly 100 per cent. of success, the same proportion is not always obtained in adult animals, although their relative refractoriness can often be overcome by such an increase in dose, provided the tumour tissue is in the positive phase of growth. Young mice which prove refractory to such large doses also prove refractory on subsequent inoculation, as also do adult and old animals.

The nature of the tumour tissue inoculated is of great importance. Where small doses give a maximum success, large doses may also do so, producing very much larger tumours in the same time; on the other hand, the larger doses may be less successful than the small ones, this being explained by the extent to which the simultaneous absorption of tumour tissue hinders growth. The absorption of tumour tissue is inversely proportionate to the number of cells growing in the graft. Whereas a large dose of cells in the positive phase leads to large tumours and no immunity, a large dose of cells in the negative phase* yields very few tumours, permits of much absorption, and gives a high proportion of resistant animals. There is much talk about the "virulent" and "avirulent" nature of different tumours, but the recognition of the fact that the "virulence" of the cells of a single tumour fluctuates between negative and positive phases will explain the contradictory statements of those who have succeeded and those who have failed to induce immunity by the injection of living tissue. It also explains the unexpected positive results obtained in some curative experiments with immune sera and the absence of any effect in others. Great care is necessary in accepting such positive results as "cures" artificially effected, unless spontaneous absorption has been excluded. For example, two tumours out of 12 disappeared after one or two injections of rabbit serum. Spontaneous absorption occurred in the same proportion of the control animals. The injections had no effect on tumours of other series in which no spontaneous absorption took place.

The phenomena discussed in the preceding pages point clearly to the interaction of two independent factors: (1) the variations from mouse to mouse of the susceptibility to (or suitability for) the growth of cancerous grafts; and (2) the variations in the energy of growth of the tumour cells.

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* *Cf.* protocol, p. 178, and footnote.

The first variable fluctuates between fairly wide limits in individual animals, but when large numbers of young mice are compared together, their resistance acts like a sieve, with unequal meshes, it is true, but, on the whole, of the same average fineness.

Spontaneous tumours vary considerably in the ease with which they can be propagated, *i.e.*, with which they can pass the sieve. These differences have been regarded as depending on differences in "virulence." There is some evidence to show that the sporadic tumours fluctuate in energy of growth just as do the propagated tumours,* and the direct conclusion as to "virulence" from one primary transplantation may be upset when the same spontaneous tumour gives an opposite result, *i.e.*, is again transplanted after recurrence. The term "virulence" is therefore unfortunate, and must be used with so much reservation that it would be better discarded altogether.†

The influence of the individuality, *i.e.*, the sum total of changes due to the past life of the organism, will be to make any mouse different from all others, and these differences will increase the longer the animal lives. The difficulty of obtaining success in the primary transplantation of spontaneous tumours would be accounted for by supposing that the new animals provide an environment to the cancer cells so strange, that they cannot survive the interruption to their nutrition. Their failure to grow does not necessarily imply that they would fail to proliferate in their new hosts if the conditions to which they had been accustomed could be immediately supplied in the experiment. Cells which have lived and become accustomed to the body fluids of one mouse for, say, two years, may easily die or fail to adapt themselves when transferred to the bodies of new animals. The frequency, in our experience, of large metastases in animals spontaneously affected, is in marked contrast to the difficulties in obtaining growth in normal animals, and harmonises well with this view. What has been said of the differences in transplantability between spontaneous tumours appears to hold also with reference to separate strains of propagated tumours. If the resistance of the mice be made the criterion for the energy of growth of sporadic tumours, the same standard is equally applicable to the continued propagation of a single tumour. It is impossible to imagine that the differences brought out by our experiments on the fluctuations in energy of growth are due entirely to variable susceptibility of the animals. The simultaneous appearance of numerous spontaneous absorptions, and associated therewith the rapid diminution in the percentage of success in parallel experiments,* can only be referred to fluctuations in the energy of growth of the parenchyma cells. Any other explanation, and especially an explanation by assuming increased resistance of the animals, introduces an arbitrary assumption which further complicates the conceptions.

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* *Vide* 'Roy. Soc. Proc.,' B, vol. 78, 1906, p. 220. Graphic record of transplantation of a sporadic tumour (XIX) which recurred three times after partial excision.

† *Vide* 'Sci. Report II,' Part 2, p. 40; Ehrlich, 'Berl. Klin. Woch.,' No. 28, 1905, where he also proposes "energy of growth" as an alternative.

The resistance of the animals can be altered, and this change has been described as an active immunity. We shall continue to speak of resistance or refractoriness. The absorption of tumours which have grown for a time undoubtedly calls forth this alteration in a high degree. The absorption of inoculated material without obvious tumour formation has similar consequences. Increased resistance results when blood injected subcutaneously is absorbed. The refractory condition induced in this way is not so perfect as that following the absorption of tumour material. It is, however, perfectly definite, and removes any cogency which might attach to the argument that the induction of a refractory condition by absorption of growing tumour or living tumour cells indicates the existence of a hypothetical cancer virus. The evidence we have obtained that the blood of other animals is unable to induce such an alteration in mice, points to the same conclusion. The absorption of the tumours of animals of alien species when inoculated into mice is likewise devoid of effect.

All these actions are curiously limited to the production of an insusceptibility to subsequent transplantation. We have not been able to induce such an alteration in animals with growing inoculated tumours that they should become unsuitable for growth to continue once it has started. It would appear that the negative phase in growth of the tumour cells plays the principal *rôle* when spontaneous absorption occurs, the fall in the energy of growth and the resistance of the mouse together contributing to the final absorption. Once the cellular graft has been vascularised it is in a much stronger position with reference to conditions which may be unfavourable to it, than is a graft in the days immediately following transplantation. This is much more vulnerable.

The biological reactions described in this paper are effective by means of the body fluids. They are analogous to those that are now well known as specific hæmolytic, cytolytic, and precipitin reactions. As pointed out in Second Scientific Report, 1905, pp. 32—33, they are of far greater delicacy, and have only been revealed by using living cells as indicators.

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* 'Roy. Soc Proc.,' B, vol. 78, 1906. Graphic record on p. 206, and protocol, p. 209.

The experiments recorded in this paper give further evidence of the great delicacy and the very narrow range of variations in the soil furnished by the living animal, within which growth can proceed or is inhibited. Growth has only been hindered by the intervention of mouse tumour or normal mouse tissue, to an infinitesimal degree by the normal tissues of the rat, and not at all by those of less nearly-related animals. We therefore feel justified in concluding that these reactions are parallel to those others whereby blood relationship has been established (Uhlenhuth).

When we attempt to formulate an explanation of the nature and mechanism of the changes leading to inhibition of growth, it must be clearly kept in mind that the evidence is almost entirely in the direction of showing an alteration in animals whereby they are unsuitable for or inimical to the establishment of grafts. There is no satisfactory evidence of an induced action on growing vascularised tumours, but only one against newly-introduced grafts. Spontaneous absorption, depending largely on alterations from the side of the parenchyma, is sufficiently frequent to enforce caution in assuming an artificial alteration in the resistance of animals already carrying growing tumours. Complete absorption is necessary to induce absolute refractoriness to subsequent inoculation. Hence the action of various procedures in inhibiting the growth of grafts is not necessarily or even probably due to a direct action on the parenchyma cells.

The connective tissue reaction, which a cancerous graft elicits, is taken advantage of by the cancer cells and acquires characters specific for each sporadic tumour. It is immaterial whether this reaction is protective on the host's part, and only not effective because the slight differences between the tumour cells and normal mouse tissues* do not sufficiently stimulate the protective process. The necessity which exists on the part of the cancer cells for this reaction if they are to continue to grow, renders it possible that a refractory condition may indeed not have anything to do with an action against the cancer cells, but, on the contrary, be due to an alteration of the connective tissue of the host which hinders it from supplying the necessary connective tissue reaction. The possibility must also be entertained that the alteration is directed against the chemotactic influence which may be presumed to be exerted by the cancer cells on the connective tissue of the host. Our own experiments have not yet given satisfactory direct evidence of an anti-toxic or anti-cellular action, but we are far from denying the possibility that such an action may be obtained.

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* Once the tumour cells are dead they excite an energetic phagocytosis. *Cf.* figs. 43—48, 'Second Scientific Report,' Part 2, 1905.

The phagocytosis of formed cellular elements plays an important *rôle* in inducing resistance; serum is impotent to produce resistance, blood corpuscles do so. The energetic phagocytosis which accompanies the spontaneous absorption of transplanted tumours, and which occurs in absorption after exposure to radium, speaks strongly for the conclusion that the processes are the same in kind when blood or tumour cells, being absorbed, produce resistance. But we are as yet unable to determine the extent to which agencies directed against the tumour cells themselves may assist in determining their early death in protected animals. Other experiments still in progress may be expected to clear up the relative importance of the parts played by the hypothetical inhibition of the specific stroma reaction, or of an equally hypothetical direct lethal action on the tumour cells.

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On the Combining Properties of the Opsonin of an Immune Serum.[*]

By Robert Muir, M.D., Professor of Pathology, University of Glasgow, and W. B. M. Martin, M.B., Carnegie Research Scholar.

(Communicated by Dr. C. J. Martin, F.R.S. Received January 18,—Read February 7, 1907.)

In a recent publication[†] we have studied the combining properties of the thermolabile opsonins of normal sera, and have shown that various substances or combinations of substances which absorb serum complements also absorb the opsonins in question. Of special interest is the fact that red corpuscles, and also heated normal sera, which by themselves have no appreciable effect either on complements or on opsonins, absorb or fix both of these bodies when combined with their corresponding anti-substances, immune-bodies and precipitins respectively. Since the time of this publication we have extended our experiments, and have always obtained the same result—the opsonin is always fixed when the complement is fixed. Using the term complement in the bio-chemical sense, we have said that the normal thermolabile opsonins belong to the group of complements. And we have also found that just as complements in their combining affinities do not possess specific properties, but are absorbed by a great many different substances, so also these thermolabile opsonins show a corresponding community in their combining relationships. We, however, expressly left out of consideration the special opsonins of immune sera, and also the thermostable opsonins of normal sera, of which latter the stable opsonin of human serum for the diphtheria bacillus may be taken as an example. In the present communication we shall consider the thermostable opsonins of immune sera.

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[*] Towards the expenses of this research a grant was received from the Carnegie Trustees, for which we have pleasure in recording our indebtedness.

[†] Muir and Martin, 'Brit. Med. Journ.,' 1906, vol. 2, p. 1783.

It is unnecessary to enter in detail into the literature of the subject, as this has been already done by Dean.* It is, however, advisable for the sake of clearness to refer to the chief facts which have been established and to the chief points at issue. It is now a considerable time since Metchnikoff showed that the establishment of active immunity towards various bacteria was often accompanied by increased phagocytic action on the part of leucocytes and other cells, or by the appearance of phagocytic action when this was absent under natural conditions. Denys and Leclef† showed in the case of rabbits immunised against streptococci that the increased phagocytosis was due not to changes induced in the leucocytes, but to an alteration in the serum, and pointed out that the leucocytes of the immune animal when placed in a normal serum showed no greater phagocytic activity than normal leucocytes did. Wright and Douglas‡ were the first to show that phagocytosis by leucocytes in the presence of normal serum depended upon certain thermolabile substances—"opsonins"—in the serum which became fixed to the bacteria in question and made them a prey to the leucocytes. Their results were confirmed by Bulloch and Atkin,§ by Hektoen and Ruediger,‖ and by others, and may now be accepted as established beyond question. We have, on the other hand, a large group of observations which show that in *immune sera* the substance which leads to the phagocytosis of bacteria, or of red corpuscles, as the case may be, is thermostable, *i.e.*, resists a temperature of 55° C. for an hour. Among such observations may be mentioned those of Savtchenko,¶ Neufield and Rimpau,** Dean,†† Leishman,‡‡ and others. And Wright and Reid§§ have shown that in certain cases the serum of patients suffering from tuberculosis may contain a considerable proportion of heat-resisting opsonin, a circumstance which may aid the diagnosis. The question therefore arises as to what is the relationship between the thermolabile and the thermostable opsonins. Dean thinks that the opsonin is of the nature of an immune substance, and that in the case of normal serum it undergoes a large fractional destruction by heat, whereas in an immune serum the portion which is thermostable is increased. He, however, mentions the possibility that the opsonic effect of a normal serum may in part be due to complement. Wright and Reid consider that the opsonin in a normal and in an immune serum alike is one and the same, and prefer to call it thermolabile. The question as to the identity of such substances may be studied in other ways than that of testing their powers of resistance to heat. One method is to test whether cell receptors combined with their corresponding immune-bodies will take up the opsonins of immune sera, as we have shown that they take up the labile opsonins of normal sera and as they take up complements. Another method is to test the degree of specificity in their combining affinities—to test, that is, to what extent one bacterium will absorb the opsonins for other bacteria. This has been done by Bulloch and Western* to a certain extent, and also by Hektoen† in the case of hæmopsonins, and to these experiments further reference will be made below. But the method has not been carried out fully in a comparative way as between normal and immune sera respectively.

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* Dean, 'Roy. Soc. Proc.,' vol. 76, p. 515.

† Denys and Leclef, 'La Cellule,' 1895, p. 177.

‡ Wright and Douglas, 'Roy. Soc. Proc.,' vol. 72, p. 357.

§ Bulloch and Atkin, 'Roy. Soc. Proc.,' vol. 74, p. 379.

‖ Hektoen and Ruediger, 'Journ. of Infectious Diseases,' 1905, p. 128.

¶ Savtchenko, 'Annales de l'Inst. Pasteur,' 1902, p. 106.

** Neufield and Rimpau, 'Deutsch. Med. Wochenschr.,' 1904, p. 1458.

†† Dean, *op. cit.*

‡‡ Leishman, 'Path. Soc. Trans.,' 1905.

§§ Wright and Reid, 'Roy. Soc. Proc.,' vol. 77, p. 211.

We shall now give an account of experiments performed by us to elucidate these questions in the case of an immune serum, giving in each instance for comparison the effects on normal opsonins under the same conditions of experiment. We have made use of two samples of anti-staphylococcic serum. One of these was kindly given to us by Dr. Dean, for which we have pleasure in recording our indebtedness, whilst the other was obtained by us from a rabbit by repeated intravenous injections of dead cultures of *Staphylococcus aureus*. As it was employed at various stages in the process of immunisation, the degree of the opsonic effect varies considerably in the different experiments. We may state that heating for several hours at 55° C. appears to have no appreciable effect on the thermostable immune opsonin. The serum thus contrasts very markedly with the normal rabbit's serum, the opsonic effect of which is practically destroyed by heating for an hour at 55° C.

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* Bulloch and Western, 'Roy. Soc. Proc.,' vol. 77, 531.

† Hektoen 'Journ. of Infectious Diseases,' 1906, p. 721.

I. THE EFFECTS OF ABSORBERS OF COMPLEMENT ON NORMAL AND IMMUNE OPSONINS.

We have tested whether or not the three combinations of receptors + immune-body which we formerly used, absorb the opsonin of the immune serum.

The two following experiments bring out at a glance the differences in the effects of treating a normal and an immune-serum in various ways, each serum being afterwards tested on an emulsion of the *Staphylococcus aureus* in the usual way :—

	Opsonic index.*
Normal serum of rabbit—	
Fresh and untreated	18·1
Heated one hour at 55° C.	0·6
Unheated and treated with red corpuscles + immune body	0·7
Unheated and treated with serum precipitate	1·0
Unheated and treated with emulsion of *Staphylococcus aureus*	0·7

It is thus seen, in accordance with our previous results, that practically all the normal opsonin is removed by these various methods of treating the serum. To contrast with this we give the results in the case of an immune serum.

	Opsonic index.
Anti-staphylococcus serum from rabbit—	
Fresh and unheated	12·3
Heated one hour at 55° C.	2·9
Unheated and treated with red corpuscles + immune body	3·1
Unheated and treated with serum precipitate	3·5
Unheated and treated with emulsion of *Staphylococcus aureus*	0·7

It is thus seen that† treating the serum with the substances which absorb complements has practically the same effect on the serum as heating has; there being approximately the same amount of immune opsonin left. An emulsion of staphylococcus, however, removes the opsonin almost entirely.

Other confirmatory examples may be given :—

Treatment by Precipitate.

	Opsonic index.
Anti-staphylococcus serum—	
Fresh and unheated	18·2
Heated one hour at 55° C.	6·9
Unheated and treated with precipitate	5·8

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* The term "opsonic index" is used in the original sense to indicate the average number of cocci contained in a polymorphonuclear leucocyte. The observations given in each table were of course carried out at the same time.

† The methods of treating a given serum are the same as those given by us in our previous paper, quoted above.

In this case there was an enormous excess of precipitate, the actual bulk of the precipitate being three times that of the serum; there would, therefore, probably be some dilution of the serum. The treated serum has practically the same opsonic value as the heated.

*Treatment by Precipitate.**

	Opsonic index.
Anti-staphylococcus serum—	
Heated at 55° C.	7·2
Heated and treated with precipitate	7·0
Normal serum—	
Unheated	24·6
Unheated and treated	0·86

Treatment by Red Corpuscles + Immune-Body.

Anti-staphylococcus serum—	
Heated at 55° C.	8·3
Heated and treated	9·4
Normal serum—	
Unheated	20·8
Unheated and treated	0·8

The opsonin of the normal serum is absorbed, whereas that of the immune serum is unaffected.

Treatment by Emulsion of V. Metchnikovi + *Immune-Body.*

	Opsonic index.
Anti-staphylococcus serum—	
Heated at 55° C.	17·2
Heated and treated	17·5
Normal serum—	
Unheated	25·0
Unheated and treated	2·36

The result is the same as before.

We have also endeavoured to remove the opsonin of the immune serum by treating it twice with a considerable quantity of serum precipitate, the precipitate being separated by centrifugalisation after each treatment, but no diminution of the opsonin has resulted. The following is an example in which the effects on the normal and the immune serum of the rabbit are once more compared:—

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* In this and the two following experiments the anti-serum was the one which we obtained from Dr. Dean.

Normal Serum.

	Opsonic index.
Normal serum of rabbit (unheated)	17·0
„ „ treated once with staphylococcus emulsion	1·6
„ „ „ twice „ „	0·25
„ „ „ once with serum precipitate	11·3
„ „ „ twice „ „	0·3

Immune Serum.

Anti-staphylococcus serum (heated at 55° C.)	23·3
„ „ treated once with staphylococcus emulsion	23·0
„ „ „ twice „ „	2·2
„ „ „ once with precipitate	25·0
„ „ „ twice „	23·0

The result, again, is that the serum precipitate removes the opsonin from the normal serum, but has no effect on the stable opsonin of the immune serum.

Summary.—It appears, from the above experiments, that in the case of a fresh anti-staphylococcus serum the effect of treating with substances which absorb complement is practically the same as heating the serum, there being a considerable residuum of opsonin in both cases. Further, after the thermolabile opsonin has been destroyed by heat, the stable opsonin remains practically unaffected by treatment which removes normal complement and normal opsonin. In other words, substances which we may call complement-absorbers do not fix or combine with the thermostable opsonin of the immune serum.

II. On the Relative Specificity of Normal and Immune Opsonins.

An emulsion of dead bacteria is well known to have the power of absorbing serum complements, as shown, for example, by hæmolytic tests; it also absorbs the opsonin of a normal serum as tested by the *Staphylococcus aureus.* We have accordingly tested the effect on the opsonin of our immune serum. In the following tables the comparative results are shown:—

Normal Serum.

	Opsonic index.
Normal serum of rabbit (unheated)	38·0
„ „ treated with *Staphylococcus aureus*	0·02
„ „ „ *Bacillus coli*	3·3
„ „ „ *V. Metchnikovi*	4·11
„ „ „ *Tuberculosis Bacillus*	2·6
„ „ „ *Bacillus pyocyaneus*	3·4

Immune Serum.

Anti-staphylococcus serum (heated at 55° C.)	7·8
„ „ treated with *Staphylococcus aureus*	0·36
„ „ „ *Bacillus coli*	8·8
„ „ „ *V. Metchnikovi*	7·5
„ „ „ *Bacillus tuberculosis*	7·9
„ „ „ *Bacillus pyocyaneus*	6·7

The tables show that emulsions of all the organisms tested produce a marked diminution of the normal opsonin, whereas none of them, with the exception of the staphylococcus, have any appreciable effect on the immune opsonin.

In another experiment we treated the heated immune serum twice with large quantities of *B. coli*, *B. dysenteriœ*, *Cholera spirillum*, *B. typhosus*, *Staphylococcus pyogenes albus.* In the case of the last mentioned, there was apparently a slight diminution of the opsonic power, viz., from 26·7 to 20·3—a comparatively trifling effect in view of the close relationships of the two organisms; the serum was, however, practically without opsonic effect on the *Staphylococcus albus.* In the case of the other bacteria mentioned, there was no diminution whatever of the opsonin after treatment with the organisms.

These experiments, by the method of absorption, bring out a very marked difference as regards specificity between the opsonins of a normal and of an immune serum. With the exception of the possible slight effect in the case of the *Staphylococcus albus,* we have failed to find any organism, except the *Staphyloccocus aureus,* which absorbs the opsonin from the heated anti-staphylococcus serum. In the case of the opsonins of a normal unheated serum, the result is entirely different, as is shown by the table given above. Every organism tested has absorbed large quantities of opsonin when the *Staphylococcus aureus* is used as the test for opsonic action.* At the same time, the staphylococcus appears to remove this opsonin more quickly than any of the others. We have tested to what extent it is possible to remove the opsonin of normal serum for *Staphylococcus aureus* by treating the serum twice with an emulsion of another organism, *e.g.*, the *B. coli.* The results are:—

Normal Serum.

	Opsonic index.
Guinea-pig's serum (unheated)	21·5
„ „ treated once with *Staphylococcus aureus*	1·0
„ „ „ twice „ „	0·4
„ „ „ once with *B. coli*	2·1
„ „ „ twice „	1·3

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* These results, so far as the normal opsonins are concerned, are in harmony with those of Simon Lamar and Bispham, whose paper ('Journ. of Exper. Med.,' December, 1906 p. 651) has come into our hands since the present communication was written.

There is, therefore, only a slight difference as regards absorbing powers in favour of the *Staphylococcus aureus.*

The immune serum was tested at the same time, and once more a great difference is brought out.

Immune Serum.

	Opsonic index.
Anti-staphylococcus serum (heated at 55° C.)	26·7
„ „ treated twice with *B. coli*	25·4
„ „ „ *Staphylococcus aureus*	1·6

These results, as regards the specificity of the normal opsonins, appear to be at variance with the results obtained by Bulloch and Western. They found only a slight reduction of the tubercle opsonin of normal human serum on treatment with the *Staphylococcus aureus,* and of the staphylococcus opsonin on treatment with the tubercle bacillus. As stated above, we found a great reduction of the staphylococcus opsonin on treating the normal rabbit's serum with the tubercle bacillus. The difference in the results probably depends upon the amount of the bacterial emulsion employed. In every case we used a large amount; in the tubes after centrifugalisation, the volume of the deposit of bacteria would be about a tenth of the volume of the serum, sometimes more. This is, no doubt, a large quantity, but it is to be noted that the same amounts were employed in the case of the immune serum, and no diminution of the opsonin was observed. The difference in the two kinds of serum is, therefore, very remarkable.

We have stated above that the opsonin for a particular organism appears to be more rapidly removed from a normal serum by an emulsion of that organism than of any other, whilst at the same time any bacterial emulsion will absorb large quantities of that opsonin. At present we are unable to give the explanation of that fact. We use the term "normal opsonin" for the labile substance which is destroyed by heat, but we do not know whether or not another substance is present in small amount which acts as an immune body. The problem is very much the same as in the case of bactericidal action. A closely analogous phenomenon was demonstrated by Bordet* in the case of hæmolysis. Normal guinea-pig's serum, after treatment with rabbit's corpuscles, is deprived of its lytic action for these corpuscles; it still, however, produces lysis of pigeon's corpuscles. If, however, the serum be treated by a more powerful absorber of complement, viz., rabbit's corpuscles treated with immune-body, it loses also its hæmolytic action on pigeon's corpuscles. And it has been shown in our previous paper that an emulsion of a bacterium treated with immune-body absorbs more opsonin than the emulsion untreated, the amount of opsonin left being practically the same as when the serum is heated. The following is an additional example:—

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* Bordet, 'Annales de l'Inst. Pasteur,' 1901, p. 317.

Opsonic Action of Normal Human Serum on the Tubercle Bacillus.

	Opsonic index.
Normal serum unheated	6·5
,, ,, heated at 55° C.	0·2
,, ,, treated with emulsion of *V. Metchnikovi* + immune-body	0·19

III. The Relations of the Opsonin of an Immune Serum.

Our results, as above detailed, show that the opsonin of the anti-staphylococcus serum corresponds as regards specificity and combining relationships with true *anti-substances* developed in the process of immunisation. Does it correspond with the type of an immune-body or of an agglutinin ? Does it in combination with its corresponding receptor lead to the absorption of complement, or does it not ? In the first place, it is certain that every immune-body does not produce an opsonic effect. We have an anti-coli serum, for example, which contains immune-body in considerable quantity, but it has no opsonic effect. A given amount of emulsion of the *B. coli* alone was found to take up 0·04 c.c. of guinea-pig's complement, while the same amount of emulsion treated with the anti-serum took up 0·12 c.c., the absorption of complement being tested by the usual hæmolytic methods. In the case of the anti-staphylococcic serum the amount of immune-body appeared to be considerably less. The sample with the greatest opsonic power gave the following results:—emulsion of staphylococci alone took up 0·03 c.c. of guinea-pig's complement, the same quantity treated with immune-body took up 0·075 c.c. This sample had a very powerful opsonic action, giving, when heated, an average count of 20 cocci per leucocyte in the usual test. The anti-staphylococcus serum which we got from Dr. Dean had a weaker though still very decided opsonic action, yet it produced a scarcely perceptible increase in the amount of complement absorbed; that is, it contained a mere trace of immune-body. We are inclined to think from these results that the opsonin in an immune serum may not be an immune-body, but has the constitution of an agglutinin. But this is merely an expression of opinion, as we know nothing with regard to the real amounts of these substances. In order to say that the immune-body did not act as opsonin, we would need to get an anti-serum with opsonic effect but containing no immune-body, and this we have not yet obtained.

If the opsonin has the constitution of an agglutinin, that is, possesses merely a combining and an active (agglutinating) group, is the opsonin merely the agglutinin? To this question we can give no definite answer. We have tested three anti-sera, viz., the *Staphylococcus aureus*, the *B. coli*, and the *Vibrio Metchnikovi*, and have found that they all possess agglutinating properties, while the anti-coli serum alone has no opsonic action. It is quite possible that the same substances may act at the same time as agglutinin and as opsonin, and that one of these properties may be wanting in any given case; but at present we have no facts to justify any expression of opinion.

So far as phagocytosis is concerned, the all-important factor in active immunity would appear to be the development of an immune opsonin with comparatively specific characters. In testing the opsonic effect of an unheated immune serum the result will represent the sum of the actions of the complement-like opsonin and the immune opsonin. As complements do not appear to increase in amount during immunisation, a rise of opsonic index will probably depend upon the development of immune opsonin, and will thus have a certain specific character. Thus Bulloch and Western found that inoculation of a patient with tubercle vaccine produced an increase of tubercle opsonin but not of staphylococcus opsonin, and conversely inoculation with staphylococcus vaccine caused an increase of the staphylococcus opsonin but not of the tubercle opsonin. This appears to be in harmony with the results which we have obtained. On the other hand, a fall in the opsonic index might be due to diminution of the complement-like opsonin, and thus be without specific significance. Further observations will be made on this point.

General Conclusions.

(1) The thermolabile opsonin of a normal serum and the thermostable opsonin of an immune serum are two distinct classes of substances. In addition to differing markedly as regards their resistance to heat, they differ in their combining relationships.

(2) The thermostable opsonin of the anti-serum investigated is a true anti-substance, and possesses the comparatively specific characters of anti-substances in general; it is left undetermined whether it has the constitution of an agglutinin or of an immune-body, though certain facts point in favour of the former.

(3) Emulsions of other organisms other than the organism used in immunisation (*Staphylococcus aureus*) do not absorb the immune opsonin; on the other hand, they absorb large amounts of the normal complement-like opsonin.

(4) Powerful complement-absorbers—red corpuscles or bacteria treated with immune-body, or serum precipitate—have no effect on the thermostable immune opsonin, whereas they remove almost completely the labile opsonin of the normal and the immune serum alike.

A General Consideration of the Subaërial and Fresh-water Algal Flora of Ceylon. A Contribution to the Study of Tropical Algal Ecology. Part I.—Subaërial Algæ and Algæ of the Inland Fresh-waters.

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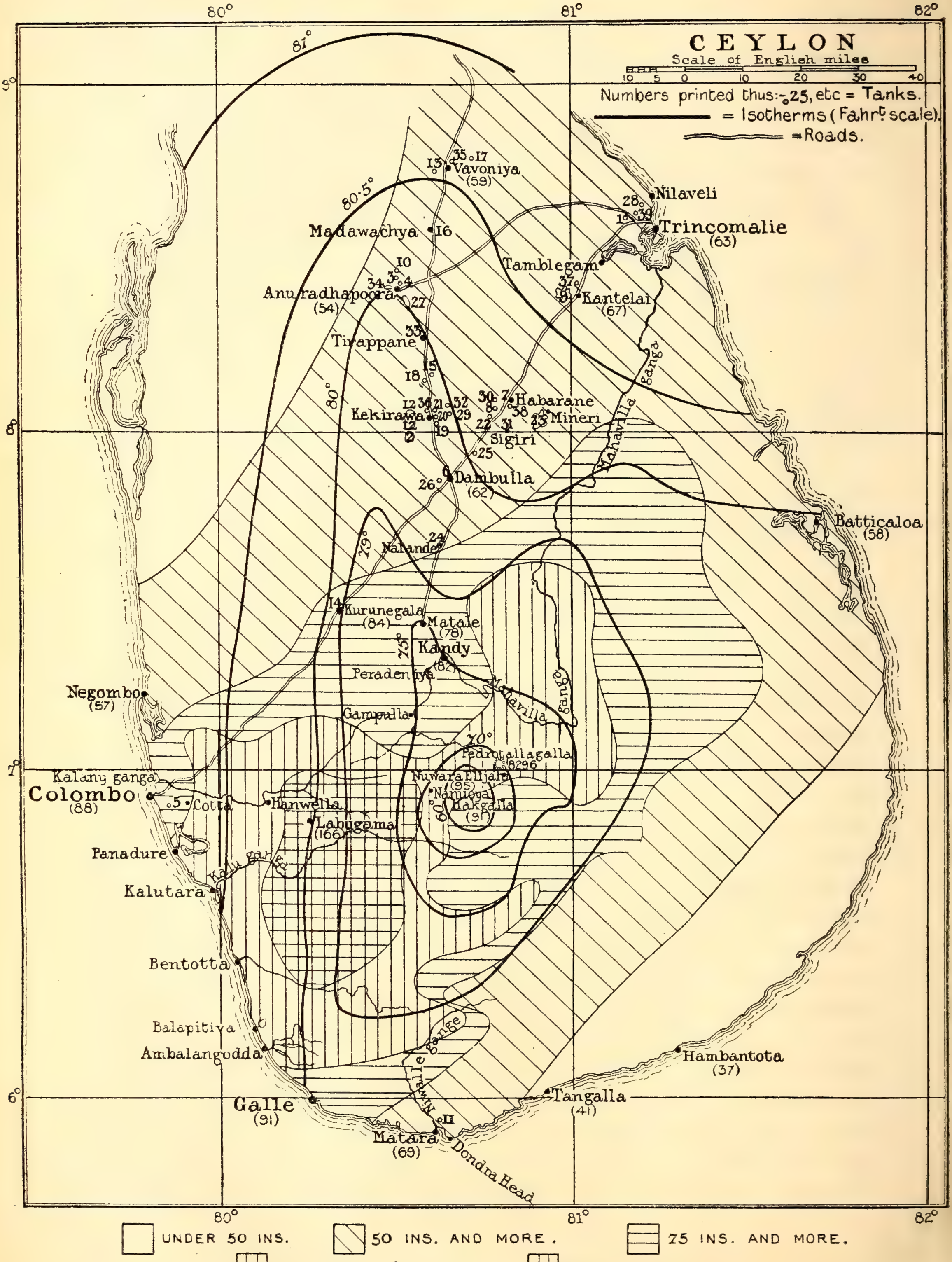
(Communicated by Professor F. W. Oliver, F.R.S.,—Received June 26,—Read November 1, 1906,—Received in revised form, January 2, 1907.)

Introductory Remarks.

During the year 1902 I was occupied with a study of the algal flora in the hot-houses of the Royal Botanic Gardens at Kew, and certain observations made then seemed scarcely to support the prevalent view of the very close similarity between tropical and temperate algal vegetation. This stimulated me to undertake a personal investigation of some tropical algal flora, and Ceylon was chosen owing to the diversity of climatic conditions which it presents. My object was rather to study the ecological and biological aspects of the algal vegetation than to make a systematic collection of materials for a flora, and I hope to be able to show that I have been in some measure successful. A careful analysis, as I think the subsequent pages of this paper will show, brings to light very considerable differences between algal growth in the tropics and in our parts, although a certain similarity between fresh-water Algæ all over the surface of the earth (considerably greater than in the case of the terrestrial flora) is indisputable. It is, in fact, a natural consequence of the uniform character of the surrounding medium.

The island of Ceylon is peculiarly favourable for a study of the influence of tropical temperature and moisture on the distribution of algal growth, since the rainfall varies considerably in different parts of the island, while the mountainous southern portion (attaining a height of 8000 feet and more) produces a very considerable range of temperature, from the intense heat of Colombo and other lowland places to the high-lying Nuwara Eliya, where, at certain times of the year, the thermometer falls to below freezing point at night. In fact, we have here confined in a narrow compass almost all the different possibilities that can be realised in the tropics. It may be well at this point to briefly outline the range of variation in this respect (see also the map). The low country, with its high tropical temperature, shows great diversity in the amount of rainfall; so that some localities are exceptionally


CEYLON
Scale of English miles
10 5 0 10 20 30 40
Numbers printed thus:-₀25, etc = Tanks.
= Isotherms (Fahrᵗ scale).
=Roads.
80°
81°
82°
9°
8°
7°
6°
81°
80·5°
80°
79°
75°
70°
60°
Vavoniya
(59)
13
35
17
Nilaveli
28
19
39
Trincomalie
(63)
Madawachya
16
10
34
3
4
Anuradhapoora
(54)
27
Tamblegam
37
9
Kantelai
(67)
Tirappane
33
15
18
Kekirawa
12
36
21
32
20
29
1
2
19
30
7
8
Habarane
38
23
Mineri
22
31
Sigiri
25
Dambulla
(62)
26
6
Mahavilla Ganga
Batticaloa
(58)
Nalande
24
14
Kurunegala
(84)
Matale
(78)
Kandy
(82)
Peradeniya
Mahavilla Ganga
Negombo
(57)
Gampalla
Pedrotallagalla
8296
Nuwara Eliya
(95)
Nanuoya
Hakgalla
(91)
Kalany ganga
Colombo
(88)
5
Cotta
Hanwella
Labugama
(166)
Panadure
Kalu ganga
Kalutara
Bentotta
Balapitiya
Ambalangodda
Nilwalle Gange
Galle
(91)
Matara
(69)
11
Dondra Head
Tangalla
(41)
Hambantota
(37)
UNDER 50 INS.
50 INS. AND MORE.
75 INS. AND MORE.
100 INS. AND MORE.
150 INS. AND MORE.

AVERAGE ANNUAL RAINFALL.

DESCRIPTION OF THE MAP.

The Map shows the different localities mentioned in the present paper, the distribution of rainfall, and the isothermal lines (after Trimen and Wright). The bracketed numbers placed below the place-names are amount of rainfall in inches. The unbracketed numbers show the position of the tanks, which are as follows :—

1. Andankulam.
2. Balaluwewa.
3. Balankulam.
4. Basawakkulam.
5. Borlasgamawewa.
6. Dambullawewa.
7. Habaranewewa.
8. Hiriwadunnewewa.
9. Lake Kantelai.
10. Karambewawewa.
11. Kekunadurewewa.
12. Kelawewa.
13. Koilpuliumkulam.
14. Kurunegalawewa.
15. Madakanawewa.
16. Madawachyawewa.
17. Madokotaikulam.
18. Mahakadawellawewa.
19. Mahakekirawa.
20. Malawewewa.
21. Mancadawewa.
22. Megaswewa.
23. Lake Mineri.
24. Nalandewewa.
25. Namoluwewa.
26. Neravieawewa.
27. Nuwarawewa.
28. Periyakulam.
29. Punchikekirawa.
30. Senadiniyagawawewa.
31. Sigiri tank.
32. Tibbotuwawewewa.
33. Tirappanewewa.
34. Tissawewa.
35. Villamkulam.
36. Walikulam.
37. Wendrenkulam.
38. Yaka-anaguhuwewa.
39. Kannia Hot Springs.

wet (*e.g.*, Kalutara, Labugama), others (under the prevalent tropical conditions) very dry (*e.g.*, Hambantota, Vavoniya), whilst numerous intermediate conditions between the two extremes are found (*e.g.*, at Colombo, Matara, etc.); the wettest place I visited was Labugama (166 inches). The amount of rainfall at Peradeniya is not much less than at Colombo, but the lower temperature, due to the higher altitude, has some influence on the algal vegetation. In travelling upwards to Nuwara Eliya the gradual change of temperature is very perceptible; the climate in the uplands is indeed practically European, although not so cold in winter, and considerably hotter in the middle of a sunny day (mean annual temperature a little over 60° F.). The rainfall, however, is heavy (about 90 inches), and that produces an abundant vegetation. If we further bear in mind that there is a coastal and an inland region, we shall have roughly reviewed the diversity of climatic conditions obtaining in the island.*

Since my stay in the island was of brief duration (August 21 to November 10, 1903) my account of the algal vegetation is necessarily very incomplete in places. I rarely stayed more than two or three days in any one locality, so that although able to familiarise myself with a wide range of climatic conditions and their influence on algal growth, I was unable to enter into detailed study of any given problem. Moreover, my observations, of course, merely deal with one phase of the algal vegetation, and it is possible that many other peculiarities would come to light if the period of observation could be extended over an entire year. I am, however, inclined to think that algal growth in the tropics will not show so marked a periodicity as obtains in our parts. The rice-fields alone are likely to be interesting from this point of view (see p. 242), since the Algæ in them are exposed to regularly alternating periods of desiccation and inundation. The greater part of my observations were carried out during the dry season, although I remained long enough to see some of the effects of the heavy rains, which begin in November. In many respects I was fortunate in being able to collect during the dry period, since the rains would very soon make access to many of the pieces of water studied exceedingly difficult. The dry season is, moreover, the interesting one from our point of view, for we then have a very essential factor influencing tropical algal vegetation in an acute stage, viz., the risk of desiccation under the burning glare of the tropical sun.

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* My travels in Ceylon may be subdivided into three distinct tours: (i) Along the western and southern coasts as far as Hambantota, the time being devoted to a study of the algal flora and Phytoplankton of the lagoons and estuaries at Negombo, Panadure, Kalutara, Ambalangodda, Bentotta, and Matara, and to an investigation of the subaërial Algæ; (ii) Into the northern plains as far as Vavoniya and Trincomalie, where I was mainly occupied with a study of the Algæ found in the inland fresh-waters (especially the numerous tanks or irrigation-reservoirs); (iii) A brief visit to the uplands round about Nuwara Eliya, which proved particularly interesting in comparison with the observations made in the lowlands. In addition to that, I stayed intermittently at Peradeniya for about a fortnight.

The main purpose of the present paper* is to indicate some of the more important differences between the subaërial and fresh-water algal vegetation occurring in the tropics and that found in temperate regions, and also to point out the essential variations in tropical algal vegetation under the influence of diverse external conditions. Most of those who have written on the subject of algal vegetation in the tropics—and the literature is practically confined to a few short introductory remarks in the systematic floras—have emphasised its similarity to that of temperate regions,† and little or no attempt has been made at determining the differences; this is mainly due to the fact that most of the works on tropical fresh-water Algæ are the outcome of casual, and on the whole, probably very unrepresentative collections by botanical travellers whose main interest lay in other directions. No one, as far as I am aware, has attempted to study the subject from the point of view put forward in the present communication.* My object is thus to outline the general features of distribution and biology, and I have rarely spent much time on specific determinations. They would in most cases be quite valueless from the present point of view, and would, moreover, have very considerably delayed publication; and it seemed well to write this general paper before one's recollections and impressions of the aspect of the algal flora had become dimmed by the lapse of a longer period than has already passed since the work was undertaken. Certain subjects, which are dealt with quite briefly in the present communication, will be considered more fully later on. I hope ultimately to be able to publish a complete systematic list of the Algæ present in my Ceylon collections, although I prefer to postpone such a publication until I have had opportunities of further study of tropical algal floras.

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* A large part of the expenses of the present investigation were covered by a grant from the Government Grant Committee of the Royal Society, to whom I am much indebted for the help thus afforded to me. I also take this opportunity of heartily thanking all those who were kind enough to assist me in the course of these investigations, either by their extensive hospitality or by direct help during the performance of the work. I wish especially to return thanks to Dr. J. C. Willis, Mr. Carruthers, and Mr. Wright, of Peradeniya, to Mr. K. Bamber and Dr. A. Willey, of Colombo, to Mr. Nock, at Hakgalla, and Mr. Lewis, of the Forest Department. The entire illustration of this paper has been carried out by my wife.

† *Cf.*, for instance, Lemmermann, "Üb. die v. Herrn Dr. Volz auf seiner Weltreise gesammelten Süsswasseralgen," 'Abh. Nat.-Ver.,' Bremen, vol. 18, 1905, p. 143 *et seq.*

Consideration of the Algal Flora.

The following special considerations are subdivided as follows:—

(*a*) Subaërial Algæ, *i.e.*, those growing on trees, stones, walls, etc. (character and distribution dependent mainly on temperature, moisture and degree of shading):—

(i) The lowland vegetation (p. 203).
(ii) The upland vegetation (p. 215).

(*b*) Algal vegetation of the inland fresh-waters (character and distribution dependent mainly on temperature, aeration and composition of water):—

(i) Algæ of the tanks and other large inland masses of fresh-water (p. 218).
(ii) Algæ of roadside ditches and pools in the lowlands (p. 233).
(iii) Algæ of marshes and padi-fields (p. 241).
(iv) Algæ of rock-pools (p. 242).
(v) Algæ of wells and springs, *i.e.*, aerated, but standing water (p. 250).
(vi) Algæ of the small pools of the uplands (p. 252).†

This subdivision is in great part artificial, but it will be found that the character of the algal growth in many of the collections of water mentioned is on the whole sufficiently different to make such a separation useful. It must, however, be pointed out, that in the case of Algæ the delimitation of distinct formations, except as regards the two fundamental groups of marine and fresh-water Algæ, is by no means so marked a one as in the case of the terrestrial higher plants. It is often, only by a study of extreme cases, that we can draw any definite limits between one kind of algal vegetation and another, for generally numerous intermediate types are to be met with. Algæ, and probably water-plants generally, are much more plastic than terrestrial plants, and many forms of aquatics are no doubt capable of adapting themselves to a variety of modifications in their environment. But there are also amongst the Algæ certain forms which are much more susceptible than others to such variations in their environment, and which are therefore of more limited distribution, and these are the forms which will characterise certain prevailing conditions in the surrounding medium. They are the character-plants, which will help us to determine our aquatic formations, and it is requisite to study them primarily, and to determine the exact conditions which influence their presence or absence. Other species will then be found to be almost constantly associated with these character-plants, and will make up the subordinate members of the formation. Whereas amongst terrestrial plants each formation probably always has one or more character-plants, which occur in this formation only, and are absolutely distinctive of it, aquatic formations will often lack so emphatic a character; they will probably in many cases be distinguished by the way in which the different members of the formation grow together, rather than by any individual species. It even seems quite possible to imagine two algal formations of almost identical specific composition, but differing from one another biologically or physiologically; as far as I am aware, such formations are not known to occur amongst terrestrial plants.

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* Bohlin, in his 'Étude sur la flore algologique d'eau douce des Açores,' gives a somewhat analogous account of the algal flora of the subtropical Azores.

† The second part of this paper, which I hope to publish in the course of the year, will deal with the Algæ of flowing water (rivers), and of the lagoons and river-estuaries, and with the Plankton.

The preceding remarks are intended to indicate the scope for research in the field of aquatic ecology, a subject on which practically nothing is known at the present day, but the importance of which it is well to recognise at the outset of considerations like those with which we are dealing. We are almost entirely ignorant of the true factors which lead to the development of certain algal forms in one piece of water and of others in a second. Certain preliminary researches on this subject, which have been undertaken in the past years on the British fresh-water algal flora, have given a number of interesting results; some of these have been dealt with at another place, where the subject of algal formations, briefly touched on above, is considered in greater detail.* My time in Ceylon was, of course, far too limited to admit of collecting more than a few meagre observations on possible tropical fresh-water formations. Moreover it is quite impossible to fully characterise an algal vegetation unless it has been studied, at least, throughout the course of an entire year. So much seems probable, however, that a large number of the algal formations of the tropics are quite different to those of our parts, although certain of them may possibly be parallel in their nature (*cf.* especially p. 240). On the whole, Phanerogams seem likely to be of more importance as character-plants in the aquatic formations of our parts than in the tropics, where the Algæ will, probably in most cases, prove more distinctive; this suggestion is, however, merely based on recollection of the aspect of the fresh-water flora of the tropics, and careful investigation may quite disprove it.

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* See Fritsch, "Problems in Aquatic Biology, with Special Reference to the Study of Algal Periodicity," 'New Phytologist,' vol. 5, 1906, p. 149 *et seq.*

(a) *Subaërial Algæ.*

A consideration of the subaërial algal flora affords a good illustration of the existence of important differences between tropical and temperate algal vegetation.

(i) *The Lowland Vegetation.*—The first impression of an algologist after landing in Colombo, which combines tropical heat with a fairly considerable rainfall (88 inches), is the abundant algal covering on the walls of the houses, on rocks, on exposed tree-trunks, etc. A rich growth of Algæ on surrounding objects is characteristic of every really moist locality, but it is only when heat and moisture combine that we get so luxuriant a growth as in the tropics; moreover, a few minutes' walk about Colombo is sufficient to convince one that the composititon of this algal growth is one quite unlike that of temperate regions. One sees practically nothing of the *Pleurococcus*, *Hormidium*, etc., so characteristic as epiphytes in our climates, green Algæ being very inconspicuous in all exposed situations (excepting *Trentepohlia*, *cf.* below). Isolated colonies of green unicellular forms are occasionally to be found in very subordinate amount amongst unicellular Cyanophyceous (adhesive) growth, but pure green coatings are completely wanting. There are very few records in the literature of the occurrence of green subaërial Algæ in the tropics* (chiefly *Pleurococcus crenulatus*, Hansg., *P. Kützingii*, West, and *P. vulgaris*, Menegh., *Hormidium murale*, Kütz),† and none of these records give us any idea of the quantity in which these species occur; nor is it possible in the absence of accurate descriptions of the habitat, to estimate the special conditions under which these forms appear in the tropics.

The subaërial‡ Algæ of the tropics nearly all belong to the Cyanophyceæ

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* Regarding terrestrial forms of *Vaucheria*, see p. 217.

† The existing data as to the composition of algal growth in the tropics are discussed more fully elsewhere ('Annals of Botany,' vol. 21, No. LXXXII, April, 1907, p. 239 *et seq.*).

‡ The term "subaërial" algal flora is used to denote all those forms not actually growing in water. Epiphytic Algæ constitute those growing on parts of other plants (trunks of trees, leaves, etc.), lithophytes are those growing on rocky surfaces.

(Myxophyceæ) and, owing to this characteristic composition, the algal covering is for the most part of a much more sombre hue than the bright green one of our parts. This dark colour is only relieved here and there by an occasional brighter patch, due either to a growth of Mosses, which, however, do not apparently prosper very well in unsheltered situations, or to the thin bright blue-green coating of an *Oscillaria,* or of some member of the Chroococcaceæ (*sensu* Kirchner), which are often able to obtain a foothold on very smooth walls, etc., which are unsuitable substrata for other forms. *Trentepohlia,* with its reddish-yellow tufts, also forms a relatively frequent and agreeable interruption to the dark Cyanophyceous growth.

Subsequent considerations (*cf.* p. 224, *et seq.*) will show that the blue-green Algæ play a far more important part in the tropics than they do with us, and a tropical fresh-water or subaërial* algal flora obtains its stamp from the prevalence of this group. In order to realise the extent of development of the subaërial Cyanophyceæ in the tropics, it is necessary to pay a visit to a damp hot-house,† or to some hot spring,‡ where conditions of a similar nature prevail. Here also we find the green Algæ crowded out and superseded by the blue-green forms.

Most of the Cyanophyceæ require two factors for abundant development; the first is plenty of moisture, the second a sufficiently high temperature. These two factors determine the distribution and character of the blue-green subaërial growth. The importance of the former factor is, of course, perfectly obvious without much comment; it is also manifest in comparing the moist lowlands of Ceylon with the drier regions having a lower rainfall. Thus at Matara, on the south coast, and at Dambulla, in the Central Province, the decrease in the extent of the algal covering is very marked; whilst in still drier localities (*e.g.*, Hambantota and Negombo, on the coast, Anuradhapoora, in the North Central Province), houses, rocks, etc., are practically clear of any growth (or only bear small Mosses and Lichens), except in particularly favourable positions. On the other hand, the heavier the rainfall at any locality, under the same conditions of temperature, the more abundantly is the blue-green element developed; the best example of this that I met with was at Labugama (166 inches), where every available object is clothed with blue-green forms. The importance of moisture is also well illustrated in the colonisation of rugged vertical surfaces, since at first only the projections, which are the points about which moisture mainly collects, become occupied by Cyanophyceous growth.

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* The only reference I am aware of that speaks of an abundant occurrence of subaërial Cyanophyceous growth in tropical regions is one by Welwitsch ('Journ. Travel and Nat. Hist.,' vol. 1, 1868, pp. 22—36), on "The Pedras Negras of Pungo Andongo in Angola." The author describes the prolific growth of a species of *Scytonema* on the upper portions of the mountains, and also refers to another species (*Porphyrosiphon Notarisii*) as covering the sandy soil in the valley of the Cuanza River for considerable stretches (*cf.* also West and West, in 'Journal of Botany,' vol. 35, 1897, p. 303; and Warming, 'Ökol. Pflanzengeogr.,' German Edit., Berlin, 1896, p. 215).

† *Cf.* Fritsch, "Algol. Notes, IV.—Remarks on the Periodical Development of the Algæ in the Artificial Waters at Kew," 'Annals of Botany,' vol. 17, No. LXV, 1903, p. 274; also "Algæ," in "The Wild Fauna and Flora of the Royal Botanic Gardens, Kew," 'Bull. Miscell. Inform. Roy. Bot. Gards., Kew,' Addit. Series V, 1906, p. 187 *et seq.* In the *Nepenthes*-house at Kew the blue-green Algæ even settle down on the leaves of the cultivated plants (*cf.* also Schmidle, "Epiphylle Algen," etc., 'Flora,' vol. 83, 1897, p. 323 *et seq.*).

‡ See G. S. West, "On some Algæ from Hot Springs," 'Journal of Botany,' vol. 40, 1902, pp. 241—248; W. H. Weed, "Formation of Travertine and Siliceous Sinter by the Vegetation of Hot Springs," 9th Ann. Rep. U.S. Geol. Survey, 1887—88, p. 619 *et seq.*; and F. Cohn, "Ueber die Algen des Karlsbader Sprudels," etc., 'Abh. Schles. Ges. Vaterl. Cultur,' 1862, p. 35 *et seq.* The vegetation in such springs is almost entirely composed of Cyanophyceæ and Diatoms (see also Sect. *b* (v), of the present paper, p. 251).

The importance of a high temperature is also easily recognised. A visit to a wet greenhouse in our regions, which is kept at a relatively low temperature, shows us that here green Algæ and Mosses are the more successful elements in the subaërial vegetation, whilst the Cyanophyceæ, where developed, present a different aspect; compare, for instance, the growth in the Temperate and Tropical Fern Houses at Kew. The same observation can, in some respects, be made in Ceylon, as we go higher and higher up the central mountain range to Nuwara Eliya (see the discussion of the subaërial Algæ of the uplands).

Though temperature and moisture are in all probability the determining factors in the distribution of the subaërial Cyanophyceæ within the tropics, this group is provided with certain biological features, which must be regarded as peculiarly suited to the dominant factors of the environment, and enable it to exist so well under the prevailing conditions. In the first place there is a well-marked alternation of a wet and dry period,* which the Cyanophyceæ are well adapted to withstand, owing to the strongly developed mucilage investments, which are so prominent a feature of the group, and which enable them to withstand prolonged drought.† In this relation, it is interesting to notice that forms with a sheath of semi-liquid diffluent mucilage are far commoner in the uplands than in the lowlands, where the risks of desiccation are much greater (see also below).* Secondly, we find the subaërial growth of Cyanophyceæ developed in situations which are often exposed to a strong light and the presence of a second colouring matter (phycocyanin) side by side with the chlorophyll, undoubtedly suits them to an existence under such conditions. The presence of this additional pigment shifts the assimilation-maximum,† but the phycocyanin, no doubt, also acts as a kind of screen to the chlorophyll, although the exact *modus operandi* remains an open question. The great variety presented by the colour of the cell-contents in the Cyanophyceæ is familiar to all, yet we know practically nothing about the meaning of these diverse shades of pigmentation. Gaidukov‡ has experimented with *Oscillaria* from this point of view, and has shown that the colour assumed by the cell-contents is complementary to that of the supplied light, and that variation of the colour of the latter induces corresponding changes in the colour of the *Oscillaria*-filaments. So that in addition to the screening action§ of the phycocyanin it probably plays some other important part in adapting the blue-green group to varying types of illumination. The absence of any protective pigment is, no doubt, one of the main causes of the lack of green algal forms in the subaërial vegetation, although the rapid growth of the hardy blue-green element certainly contributes very considerably to crowd them out. Mosses and Liverworts are also mostly wanting in localities exposed to the strong light, although they get on well enough as epiphytes on the tree-trunks in the moist shady jungles and forests. It is interesting to notice in this connection that the only member of the green Algæ, which really competes successfully with the

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* See J. C. Willis, "The Royal Botanic Gardens of Ceylon as a Centre for Botanical Study and Research," 'Annals. Roy. Bot. Gards., Peradeniya,' vol. 1, 1901, p. 17 *et seq.*; also "Studies in the Morphology and Ecology of the Podostemaceæ," *loc. cit.*, vol. 1, Part 4, 1902, pp. 278—279.

† Hansgirg, "Ueber *Bacillus muralis*, Tomaschek, nebst Beiträgen zur Kenntnis der Gallertbildungen einiger Spaltalgen," 'Bot. Centralbl.,' vol. 35, 1888, pp. 54, 102; B. Schröder, "Untersuchungen über Gallertbildungen der Algen," 'Verhandl. Nat.-Med. Ver. Heidelberg,' New Ser., vol. 7, p. 183 *et seq.*, especially p. 185.

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* *Cf.* also Gomont, "*Myxophyceæ hormogoneæ*" (in J. Schmidt., "Flora of Koh Chang"), 'Bot. Tidsskrift.,' vol. 24, 1901–2, p. 203. He remarks: "Si l'on examine le catalogue que nous en donnons, on s'aperçoit immédiatement que les Algues à gaînes épaisses et colorées l'emportent de beaucoup par le nombre des espèces et par leur fréquence. Ainsi, tandis que les genera *Oscillatoria*, *Lyngbya*, *Phormidium*, *Hydrocoleum*, ne sont representés chacun que par une espèce, les *Scytonema* et *Stigonema* en renferment treize à eux seuls . . . cette absence n'est pas purement accidentelle, mais, ainsi qu'on l'a maintesfois observé, les plantes les mieux adaptées aux régions tropicales sont celles qui trouvent dans l'épaisseur, la consistance ou la coloration de leur gaîne une protection contre les sécheresses fréquentes et l'intensité de la lumière."

† See Pfeffer and Ewart, 'The Physiology of Plants,' vol. 1, Oxford, 1900, pp. 343—344.

‡ "Über den Einfluss farbigen Lichts auf die Färbung lebender Oscillarien," 'Anhg. z. Abh. Kgl. Preuss. Ak. d. Wissensch.,' 1902; "Weitere Untersuchungen über den Einfluss farbigen Lichts auf die Färbung der Oscillarien," 'Ber. Deutsch. Bot. Ges.,' vol. 21, 1903, p. 484 *et seq.*

§ In many cases the cell-contents no doubt obtain additional protection from the intense light in the sheaths of the filaments, which are very frequently brown, yellow, or red in colour. The colour of the sheath is also subject to much variation (*cf.* the first footnote on this page).

Cyanophyceæ in exposed situations, is the genus *Trentepohlia;* the latter, as is well known, is characterised by the presence of hæmatochrome in its cells, and this probably also has a protective function against too intense illumination. Such an interpretation is suggested by the fact that the colour of the filaments varies according to the intensity of the light, being more or less green where shaded, and yellow to yellowish-red in the full light.* Species of this genus are particularly common on the trunks of the cocoanut trees in the plantations along the sea shore and on embankments by the roadside.†

The subaërial Cyanophyceæ in Ceylon exhibit a number of different modes of growth, which are, no doubt, of the nature of adaptations to special external conditions. My stay in the island was not sufficiently prolonged to make it possible to analyse these conditions satisfactorily, but I am able to put forward a number of probable suggestions regarding the factors favouring the one or other mode of growth. The simplest and one of the commonest habits assumed by the subaërial blue-green element may be styled the "adhesive" mode of growth, the Alga being firmly adpressed to the substratum; this occurs in all the gelatinous‡ and encrusting unicellular forms (*Aphanocapsa, Aphanothece, Glœocapsa, Chroococcus,* etc., fig. 1, B and D), and also in certain of the filamentous genera (*Nostoc,* a few species of *Calothrix, Oscillaria, Hypheothrix, Lyngbya,* especially the section *Phormidium*). On the whole this type of growth rarely attains considerable thickness, a result probably of the difficulties of interchange of gases in the inner layers of the stratum. The only well-marked exception is the genus *Phormidium,* but here the inner portions of the often thick lamellæ merely consist of empty sheaths, while only the outer parts contain living filaments. The forms exhibiting the adhesive mode of growth are the first successful colonists of new ground, and are especially adapted to grow on smooth surfaces, where they often form a very thin, slippery film.

A second type of growth may be described as the "tangled" one; this (like the remaining two) is confined to the filamentous forms, and is due to the fact that the filaments are bound together in bundles, which are irregularly interwoven with one another, much as in many of the submerged filamentous Algæ. The denseness of the tangles varies very considerably. In the majority of cases, this growth was found to be relatively thin (although generally attaining greater thickness than an adhesive growth) before its surface became colonised by other kinds of Algæ (exhibiting tufted growth). In the few cases in which a thick tangled stratum was observed, only the external portion was generally found to be living, the inner part consisting largely of dead sheaths. The tangled type of growth leads to the retention of a considerable amount of air in the interspaces between the filaments, and this is probably of advantage from a respiratory point of view in comparison to the adhesive habit; this suggestion has, however, first to be confirmed by experiment. On the other hand, this mode of growth (like the adhesive one) is not well adapted to the absorption of atmospheric moisture, since only few of the filaments project freely into the air. Such growth is probably largely dependent for its supply of moisture on the substratum on which it grows, and this is in agreement with the fact that it prospers best on clay embankments or porous rock-surfaces. As it gets thicker, it very often passes over into tufted growth (see below and fig. 1, C) or becomes overgrown by the tufted growth of another more successful form.*

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* See also the statements on p. 248 of Oltmanns' 'Morph. u. Biol. d. Algen,' vol. 1, Jena, 1904.

† Species of *Trentepohlia* may become epiphyllous, like some of the Cyanophyceæ. I did not observe any such cases in Ceylon, although that is probably due to the small amount of time at my disposal. Other Chroolepideæ are permanent leaf-epiphytes.

‡ It should be pointed out, however, that these gelatinous forms are rather rare in the lowlands, and far commoner in the moist uplands (*cf.* below, pp. 215, 216).

The tangled type of growth is met with in subaërial forms of *Tolypothrix*, *Hapalosiphon*, *Scytonema*, *Stigonema*, etc., and in nearly all cases the filaments are provided with firm consistent sheaths. In most of these genera (mainly in *Stigonema minutum*, Hass. ? and species of *Tolypothrix*) one occasionally meets with tangles growing in protected situations, and exhibiting a characteristic association with a film of air which gives their surface a bluish grey or bluish colour and a silvery sheen, and not only occupies the interspaces of the tangles, but covers their entire surface. The forms under consideration seem to avoid direct moisture as much as possible, and grow in situations which are more or less protected from the rain. The air is held so firmly in the interspaces of the tangle that it has not been dislodged by the preserving fluid, but it seems probable that the impact of falling rain-drops would be able to displace it, and that this is the reason why these forms are only found growing in sheltered situations. The species exhibiting this association with air, however, possess another peculiarity in the form of an irregular incrustation on the sheath, completely absent at some points, but forming an opaque covering which obscures the enclosed trichomes at others. In some

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* It may be pointed out, however, that both from the point of view of respiration and of absorption of moisture the various species and genera of Cyanophyceæ may differ markedly among one another; thus one form may be much better enabled to withstand the drawbacks of an adhesive or tangled growth than another. That, under certain circumstances, a tangled growth may be laden with moisture is evidenced by the fact that in damp protected localities I occasionally found aquatic Algæ (*e.g.*, species of *Ulothrix*) growing on the surface of the tangle. These Algæ probably obtain the necessary moisture by absorption from the tangle below.

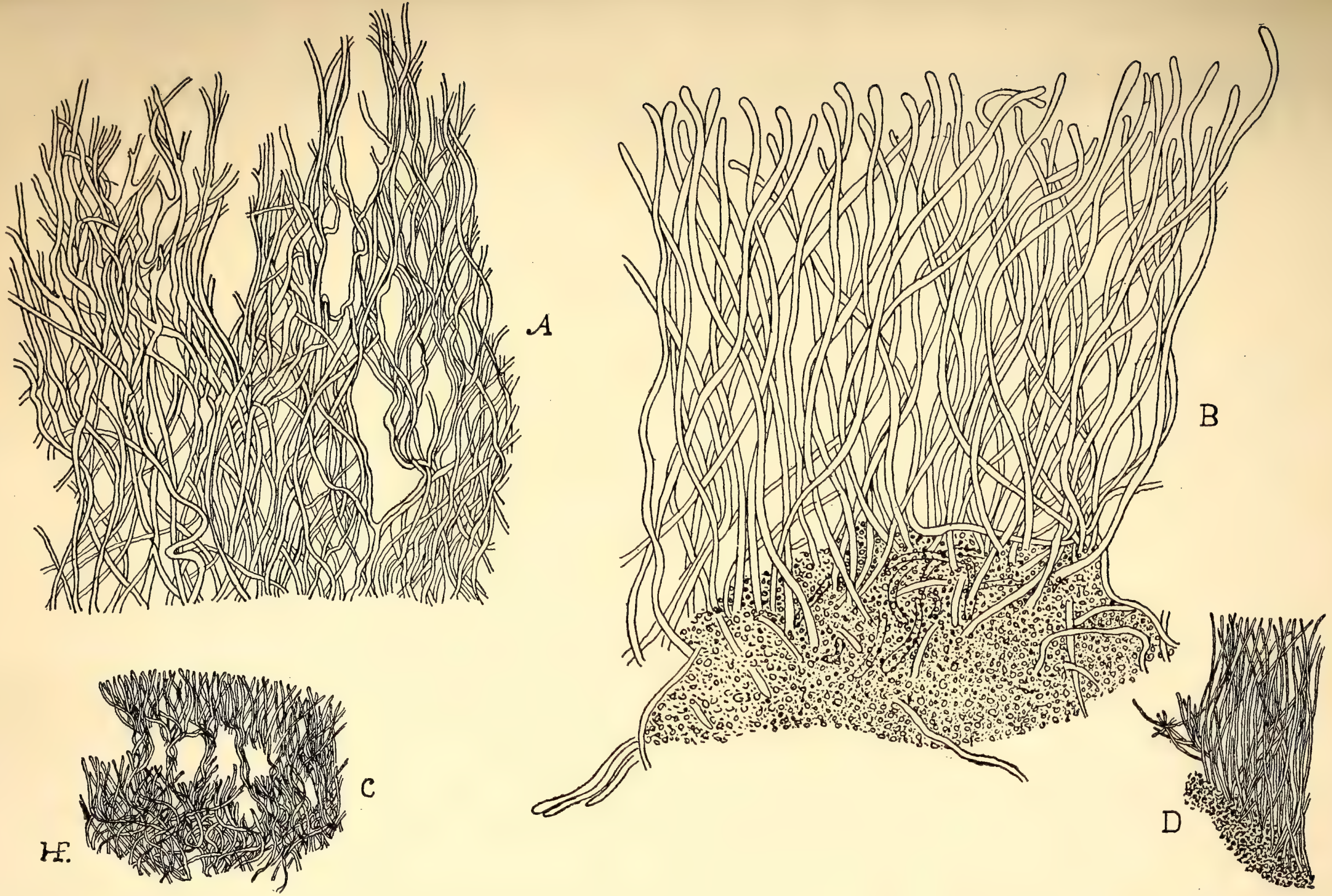

Fig. 1.—Illustration of various Types of Tufted Growth (only the sheaths of the individual filaments are shown). A (×60).—Rather irregular tufted growth of a *Tolypothrix*. B (×60).—Tufted growth of a *Tolypothrix*, showing disorganised remains of the adhesive base. C (×9).—Regular tufted growth of a *Scytonema;* upright branches arise from a basal tangle, the former then branch abundantly, the ramifications being arranged to form a fairly regular palisade-like layer. D (×12).—Dense tufted growth of *Schizothrix*, arising from an adhesive base.

cases this incrustation is due to carbonate of lime, but in other cases it is not affected by acids, and it has as yet been impossible to determine its exact nature. It seems plausible to associate this incrustation with the presence of the air, and possibly the latter may be a result of the formation of the former. The phenomenon is certainly no casual one,* as it is to be met with quite frequently in different parts of the island; the species exhibiting it are in part common forms throughout the lowlands, and are by no means always found with the associated air.

The third type of growth is the "tufted" one,† and is characteristic not only of many of the Cyanophyceæ, but also of the species of *Trentepohlia*.‡ In each tuft the filaments are firmly intertwined with one another to form bundles (fig. 1), which stand out approximately vertically from the substratum. The free ends of the filaments thus project outwards, and are occasionally considerably elongated and practically unbranched (frequently in *Scytonema* and *Tolypothrix*); sometimes the projecting ends are of a different (generally brighter) hue than the remaining parts of the filaments. The tufts are often (notably in *Schizothrix*, fig. 1, D) so closely placed that, if they all happen to be of the same length, it is difficult without the aid of a lens to determine the composite character of the growth; on the other hand, in the genus *Symploca* the tufts are generally quite isolated from one another. Where a rich tufted growth occurs, the substratum presents a velvety appearance owing to the numerous vertical tufts of intertwined filaments, and this pilose covering is like a wet sponge in the rainy season. The least pressure squeezes water out of it. The tufted type of growth constitutes the prevalent habit in *Symploca, Schizothrix* (fig. 1, D), etc., and a number of forms which assume the tangled type of growth at first, subsequently raise their filaments into tufts; such are *Tolypothrix, Scytonema* (fig. 1, C), *Stigonema*, etc., especially the species of the first-named genus varying very much in their manner of growth.

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* In a few cases, encrusting unicellular forms were found to exhibit the same characteristic association with a film of air, although not growing in protected situations; the forms involved are species of *Glœothece*. Here also the thick outer lamellæ of the sheaths in most of the colonies were provided with a thin incrustation of a substance whose nature I have been unable to determine. Under the microscope, one or more air-bubbles are seen to be associated with each colony, being frequently drawn out into a kind of beak, the apex of which is in very close connection with the sheath of the Alga.

† I must point out that the terms "tangled," "tufted," etc., refer to the macroscopic appearance of the Alga. Under the microscope, many Algæ of the adhesive type show a tangle of filaments and, in the tufted form of growth, the filaments in each tuft are entangled with one another to a varying extent.

‡ Some of the species of *Trentepohlia* exhibit a slight tangled growth at first, while the basal disc ("sole") is, of course, always adhesive.

These forms, and especially those exhibiting pure tufted growth (*i.e.*, in which there is little or no tangled base), readily absorb moisture from the substratum by capillary attraction, whilst as long as the air is relatively dry (as compared with the substratum), water will also be given off by evaporation from the outer ends of the tufts, so that a constant circulation of water will take place. On the other hand, when tufted growth occurs on a substratum which does not furnish much moisture, the outer ends of the tufts will carry on the reverse process, and absorb water-vapour directly from the air, provided the latter is sufficiently moist. The tufted type of growth is thus liable to be successful on all kinds of substrata provided the air contains the necessary amount of moisture. These suggestions as to the biological value of tufted growth seem to agree with the main facts of the distribution of the latter. We should expect not to find tufted growth or to see it restricted to well-protected situations in dry localities, in which both air and substratum furnish little moisture; and this is really the case, for at Anuradhapoora, Negombo, etc., such subaërial growth as does occur is almost exclusively adhesive or tangled.* On the other hand, the wetter the locality the more abundant does this type of growth become; even at Kew it only occurs in the very damp hothouses (*e.g.*, the *Nepenthes*-house).

Atmospheric moisture thus seems to be the chief factor determining the development of tufts, and there is good reason to look upon them as being the result of a hydrotropic stimulus (*cf.* also below). The subaërial Cyanophyceæ under discussion, although they have left the aquatic habitat, are still semi-aquatic in their requirements, and it is not surprising to find moisture influencing them in various ways (*i.e.*, general distribution and mode of growth). In moist localities (*i.e.*, where there is plenty of water-vapour in the air) the advantage of the tufted as compared with the tangled type of growth is obvious, especially in exposed situations where the surface layers of the soil are very liable to be dried up by the heat of the sun's rays, so that absorption of atmospheric moisture is imperative. In damp localities, probably, all tangled growth sooner or later passes over into a tufted one or becomes colonised by some other species with tufted growth, but in dry localities the latter affords too ready a means of evaporation and, consequently, is unfavourable. Without experimental study the conditions of respiration in this form of growth can hardly be compared with those in a tangle; in all probability, however, they are much the same in the two cases.

Some of the species of *Trentepohlia* quite conform to the above-described method of growth (*e.g.*, *T. abietina*, Hansg.), whilst others differ in the great elongation of the filaments, which hang down in long tresses (*e.g.*, *T. arborum* (Ag.) De Willd.).

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* These observations were, of course, made during the dry season; possibly during the wet period tufted growth may be more commonly developed at these localities.

The last type of growth presented by the subaërial Cyanophyceæ is really only a modification of the tufted one; it may be spoken of as "stratified." It is found in the same species as exhibit the tufted habit, although on the whole it is of rare occurrence—a result, probably, of the absence of the special conditions which lead to its development. It was mainly observed in species of *Tolypothrix* and *Scytonema*. The characteristic feature of this type of growth lies in the fact that the closely-placed tufts are arranged in regular strata forming tiers one above the other; each tier generally projects a little beyond the one above it, and in this way a habit recalling that of some species of *Hypnum** or one of Schimper's "Etagenbäume" is attained. Such growth is mainly to be found on vertical walls or tree-trunks, where the species of Alga concerned often formed huge coherent sheets, which with some little trouble could be detached in their entirety. Since this type of growth occurs mainly in shaded situations, it may be suggested that the projection of each tier beyond the one above it enables the free growing ends of the tufts to obtain the maximum amount of light; in some cases these free ends were differently coloured to the main inner mass, and these differences in coloration may be a result of the illumination (see p. 206). It seems possible, therefore, that "stratified" growth is the result of tufted growth on a vertical surface in a shaded situation, and that stratification meets the requirements of illumination combined with the largest possible extent of surface-development. But moisture may also play some part in determining this type of growth, for, if one tier of tufts is once established, others might gradually grow out below and elongate sufficiently to catch up the drops of rain running off from the tier above; in this way tier upon tier might be formed. In this connection two points may be noted: Firstly, the tufts of a tier in their entirety point downwards, so that superfluous rain-water readily runs off one tier on to the next; and, secondly, "stratified" growth occurs on substrata which probably do not furnish much water in themselves. Both illumination and moisture may thus be concerned in the development of the stratified mode of growth, but I am inclined to regard the former as more important.

A few words may be added on the localities in which these different types of growth seem to predominate, and on their inter-relation. In exposed situations in the wet regions it is usual to find only one or other of the last three types of growth (mainly the tufted one), so that ordinarily only such substrata are colonised as are suitable for their development. Tree trunks and smooth rock-surfaces, even in very wet localities, are often only provided with a very poor algal covering, since the hard bark or rock is suitable only for adhesive growth; the latter, however, probably meets with considerable obstacles in the way of establishing itself owing to the very heavy downpours of rain which scour the surface of the tree or rock. If these difficulties are successfully overcome in early stages, adhesive growth may become established, and will then, in course of time, prepare the way for other forms of growth. Decayed trunks, of course, form a much more suitable substratum and generally bear rich algal growth of various kinds. In protected situations adhesive growth becomes well developed, especially on walls, rocks, embankments, etc., and is almost invariably the forerunner of tangled and tufted growth. In many cases it is quite possible to detect the remains of an adhesive growth at the base of tangled or tufted growth (fig. 1, B, D); mostly, however, such adhesive growth is quite dead. It appears more commonly to consist of unicellular encrusting forms than of filamentous species, and the former by their decay probably afford a kind of soil for further colonisation. In the dry regions, adhesive growth is predominant everywhere. Heavy downpours of rain probably make it impossible for larger gelatinous forms to obtain a foothold except in very sheltered situations. Tangled growth, since it helps to bind the clay of an embankment together, and to make it more suited to withstand a heavy downpour of rain, is liable on such a substratum to be much more successful in exposed situations than an adhesive growth; and such embankments may furnish a foothold for tangled forms without any previous growth taking place, although in many cases there is a passing preliminary growth of the adhesive type. The tangles again give way to tufted growth, which generally gains the upper hand ultimately. This tufted growth, as already mentioned above, may belong to the same form as constitutes the basal tangle, or may be due to a distinct tufted species. Thus *Symploca* is very commonly found growing on a basal tangle of *Tolypothrix*, whilst in other places small Mosses are found arising vertically from a similar base or even from a dense tufted growth of one species or the other. In both cases I frequently found some of the filaments of the basal tangle of *Tolypothrix* using the *Symploca* or the Moss, as the case might be, as a kind of support round which they twined themselves closely (fig. 2); they thus raise their filaments out into the air and, in fact, form a kind of upright growth quite comparable to a tuft,* at

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* *E.g.*, *Hypnum* (*Hylocomium*) *splendens*. *Cf.* Goebel, 'Organographie d. Pflanzen,' Jena, 1898—1901, p. 56, fig. 27. Such growth is, of course, of quite a different nature to the case we are considering, but there is a considerable superficial resemblance.

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* This, no doubt, also illustrates the mode of formation of a tuft. One or two outstanding branches first arise from a tangle, and these serve as supports for further branches which grow up round about them.

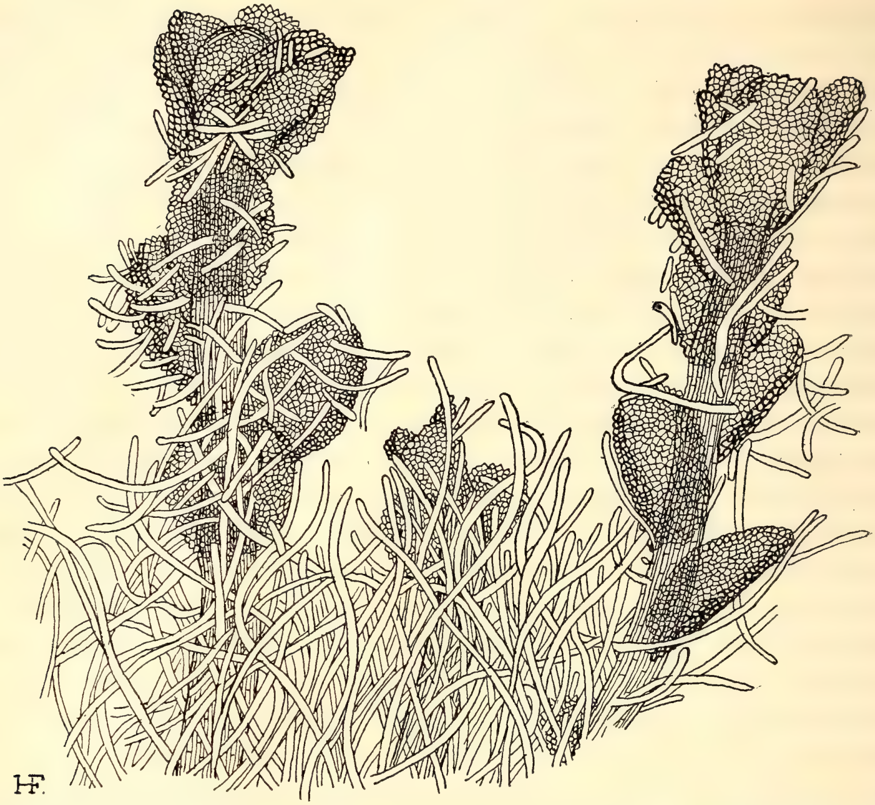

Fig. 2 (× 72).—Tufted Growth of *Tolypothrix*, colonised by Bryophytes. The Alga is using the latter as a support by means of which it raises its filaments up into the air. The free ends of these filaments are appearing at all points. Only the sheaths of the individual filaments are shown.

the expense of the *Symploca* or Moss. Such cases very clearly illustrate the tendency of the filaments of a tangle to grow away at right angles to the substratum, and the stimulus is here undoubtedly a hydrotropic one. The ultimate effect of Moss, etc., being used as a support by the Alga on which it first settles down will be to more or less kill the former, and it can only save itself by growing out more and more as it gets entwined and killed off behind. In this way a thick layer of humus will gradually accumulate and serve as a base for the development of higher plants (small ferns, etc.). This is probably the way (after preliminary adhesive and tangled growth) in which bare rock-surfaces become gradually converted into substrata suitable for all kinds of growth. The subaërial Cyanophyceæ play a great part in the progressive colonisation of bare surfaces in the tropics, and to their agency the wealth of vegetation on every conceivable object is primarily due.*

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* This subject will receive fuller treatment elsewhere (see "The *Rôle* of Algal Growth in the Colonisation of New Ground, etc.," in a forthcoming number of the 'Geographical Journal'); *cf.* also Treub, 'Ann. Jard. bot. Buitenzorg,' vol. 7, 1888, p. 213.

(ii) *The Upland Vegetation.*—The following description is based on an examination of the subaërial algal vegetation at Nuwara Eliya and neighbourhood. Although only six days (in the early part of the rainy period) were spent in this region, they were adequate for obtaining a general conception of the character of the subaërial Algæ, and I do not consider it likely that other parts of the uplands will differ very materially. Nuwara Eliya lies at a height of 6200 feet above the sea, and close at hand is Pedrotallagalla, the highest point of the island (8296 feet). The rainfall is a fairly high one (91 inches at Hakgalla) and, consequently, the subaërial Algæ are well developed, nearly every rock, tree-trunk or embankment being covered with them. This algal growth, however, bears a very different stamp to that of the moist lowlands, whilst Bryophytes and Lichens obtain a foothold, and even attain an abundant development in situations which are dominated by the blue-green Algæ in the low country. In all shaded localities, indeed, the greater part of the algal growth becomes completely obscured by Mosses and Lichens; it is best developed on smooth rock-surfaces or smooth clay-embankments in more or less exposed situations, which are apparently not so favourable for the growth of the other plants mentioned. A point which very soon strikes one with regard to the subaërial Algæ of the uplands is the great scarcity of the tufted mode of growth, so characteristic of the wet lower regions. The majority of the Algæ altogether exhibit the adhesive type, and by far the most of them are of highly gelatinous consistency. I have put together the following table with the object of showing the relative frequency of the four types of growth in the lowlands, in the country round about Peradeniya and in the uplands; the percentages are calculated from the actual number of cases examined:—*

Type of growth.	Lowlands.	Peradeniya.	Uplands.
	Per cent.	Per cent.	Per cent.
1. Adhesive	25	47·5	66·5
2. Tangled	34	35·0	26·5
3. Tufted	34 } 41	17·5	7·0
4. Stratified	7	—	—
No. of cases examined	56	40	30

The great prevalence of the adhesive habit is very manifest, and such tufted growth as occurs rarely covers any great extent of surface; tangles are rather more frequent. Embankments are generally covered with a thin slippery film of gelatinous forms (*e.g.*, the whole embankment along one side of Lady Horton's walk at Nuwara Eliya), and only at a few points are these overgrown by tangles or tufts. The Algæ (*Nostoc, Aphanocapsa, Glœocapsa, etc.*) concerned in the formation of these gelatinous films are all characterised by having very highly mucilaginous (almost diffluent) sheaths. But it is amidst the thick growth of Mosses and Lichens on the trunks of trees that these gelatinous Algæ often attain their greatest development; both on the wooded slopes of Pedrotallagalla and in the jungle on the Hakgalla rock the luxuriance of their development is remarkable. In most cases the Mosses are the more important epiphytes, but here and there gelatinous Algæ vie with them, either covering whole portions of the trunk like the exuded resin or latex of an injured tree or hanging down in long, jelly-like masses between the Mosses and Lichens.* Such growth would be almost an impossibility on a bare trunk, whilst it easily obtains a foothold and maintains its position amongst the growth of Mosses, etc. The rocks occurring in these jungles are remarkably poor in algal growth, which is probably due to the absence of forms with a type of growth suited to such a substratum. An almost diffluent *Nostoc* is not well suited for attachment to a smooth rock-surface in a rainy region, and such growth as does occur on these rocks is tangled† or very rarely tufted. The preceding remarks indicate one of the chief factors in the uplands, viz., the competition of the Bryophytes, etc.; bearing this in mind, we can understand the scarcity of tufted and tangled growth on moss-covered substrata, for it must inevitably become overgrown sooner or later by the prolific Bryophyte and Lichen vegetation.

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* The species of *Trentepohlia*, which always assume a tufted habit, are not taken into account in the calculation of the above table; their tufts are probably not dependent on exactly the same conditions as the short dense tufts of the Cyanophyceæ, for they are just as common in the uplands as in the low country.

There are thus very obvious differences between the subaërial Algæ of the lowlands and those of the uplands, but the exact factors leading to this diversity are difficult to determine. As above indicated (p. 207, footnote), gelatinous forms with semi-liquid, diffluent sheaths, do not get on successfully in the lowlands, because they are probably too susceptible to desiccation. In the part of the uplands studied, the relative humidity of the air is considerably greater than in the lowlands, whilst the temperature is, of course, lower, and these seem to be the chief factors determining the enormous development of gelatinous forms in these regions—in many localities to the practical exclusion of other types of growth. But competition, encouraging the growth of the more successful gelatinous forms, cannot always be the explanation of the absence of tangled or tufted growth; such competition can scarcely play a part on an embankment. Here it seems as though the highly mucilaginous substratum afforded by the prevailing gelatinous adhesive growth is not a suitable base for growth of a higher type. A more complete analysis of the prevailing conditions will probably cast more light on the matter.

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* The most important of these gelatinous forms are species of *Glœocapsa*, *Glœothece*, *Aphanocapsa*, *Nostoc*, and *Stigonema*. *Glœocapsa sanguinea* (Ag.), Kütz, possessing characteristic red-coloured sheaths, was particularly common on some of the tree-trunks at higher altitudes.

† In one or two cases I met with tangles associated with a film of air such as were described for the lowlands on p. 208. They are, however, of rather rarer occurrence in the uplands.

The subaërial algal flora of the uplands of Ceylon has a certain superficial resemblance to that of a damp region in the temperate zone, but it differs in its luxuriant development and in the important part played by the blue-green element. The light-conditions in particular, but also the temperature during the day (at least at most times of the year) are distinctly tropical—witness the wealth of the Phanerogamic vegetation. The green algal element, which is of considerable importance in the subaërial vegetation of our parts, is still quite crowded out by the more successful blue-green forms; their success is here again probably, to a large extent, due to adequate protection from the strong illumination. It should be noted, however, that I met with terrestrial species of *Vaucheria* (*V. sessilis* (Vauch.) DC.) at one or two points round about Nuwara Eliya—forms which, as far as my observations go, are entirely wanting in the lowlands.

The subaërial algal vegetation at places like Kandy (rainfall, 82 inches) and Peradeniya is approximately intermediate in its character between that of the lowlands and uplands, although on the whole savouring more of the former (*cf.* table on p. 215). In the wet regions of the lowlands we have a profuse subaërial flora, which is probably in the main due to the high temperature, whilst in the true uplands the rich algal growth is the result of a rather high rainfall. At Peradeniya and neighbourhood both factors are not so strongly developed, the temperature being low compared with that of the low country, and the rainfall less than at Nuwara Eliya and neighbourhood. In correspondence with this we find a marked decrease in the extent of development of subaërial algal vegetation at Peradeniya and in the surrounding country. In the wet jungles on the hillsides round Peradeniya, however (*e.g.*, at Hantane), we meet with a state of affairs rather like that on Pedrotallagalla, although on a less luxuriant scale. Otherwise the subaërial algal vegetation in these regions stands behind both that of the lowlands and uplands, and cannot even compare, for instance, with that at a place like Matara (rainfall only 69 inches !). The character of the algal vegetation is of particular interest. Tufted and tangled growths are fairly well represented (*cf.* table on p. 215)—especially the latter, which is no doubt a result of the relative dryness of the atmosphere. Gelatinous adhesive forms are much commoner than in the lowlands, although rarely exhibiting that markedly diffluent character which is such a feature in the uplands; the species of *Nostoc,* for instance, occurring round about Peradeniya, are more of the foliose type with a fairly consistent investment. The arches of the aqueduct in the experiment station at Gangarruwa, probably owing to the abundance of moisture, are exceptional, since at most points they are densely overgrown by a highly gelatinous *Nostoc* in all hues of brown and green to almost colourless.

A few words on the subaërial Algæ found on the cliffs and rocks of the sea-coast may be added, based on observations made at Matara, Ambalangodda, Bentotta, and Trincomalie. In all cases the substratum is probably subjected to occasional spray from the sea (in stormy weather), but otherwise the conditions are identical with those influencing the lowland subaërial Algæ generally. Cyanophyceæ are the prevalent forms, and especially in the higher zones their character is quite similar to that presented by the inland forms above discussed. The lower parts of the cliffs and occasional rocks on the sea-shore, on the other hand, tend to be colonised by a characteristic assemblage of unicellular blue-green forms (species of *Placoma*, *Radaisia, Entophysalis,* and *Chroothece*), which are more or less marine in character; their habitat is, no doubt, much more often reached by the sea-water, and this factor excludes the ordinary inland algal growth to a varying extent, whilst it calls forth the marine element just mentioned. The subaërial algal growth on the sea-coast finds an interesting parallel in the algal vegetation of fresh-water pools, which are found in the sand of the sea-shore at diverse points, and will be considered in Part II of this paper; in both cases the occasional advent of sea-water brings in a modifying factor.

(b) *Algal Vegetation of the Inland Fresh-waters.*

In this section I propose to deal with the macrophytic algal vegetation (both attached and floating) of the numerous pieces of inland fresh-water (excluding rivers) I had the opportunity of studying. Since the essential conditions influencing the character of the algal vegetation in these pieces of water are much the same in all cases, I shall deal with them in detail in the following discussion of the tanks, and merely point out the differences, when speaking of the other pieces of fresh-water. Minor factors cause the variation in the flora noticeable in the different collections of water.

(i) *Algæ of the Tanks and other Large Inland Masses of Fresh-water.*—The tanks are pieces of fresh-water of artificial origin, and often of very ancient date; they occur all over the island, but are especially common in the dry northern half. They have partly been made by the damming up of broad river valleys, and partly by the collection of rain-water in specially prepared hollows. In a few cases they occupy natural hollows in some of the large basaltic masses which occur at many points (*e.g.*, Nalande and Dambulla). Large numbers of small shallow tanks are found near every village in the drier parts of the island. Tanks of medium size, and apparently of some depth, are Madawachyawewa,* Kekunadurewewa, Villamkulam,* Habaranewewa, Periyakulam, Kurunegalawewa, and we may also include here the lakes at Kandy, Colombo, and Nuwara Eliya. A few of the tanks are of very large dimensions; of these I was able to examine the following (approximately in order of size, beginning with the largest): Lake Mineri, Kelawewa and Balaluwewa, Nuwarawewa, Tissawewa, Basawakkulam, Lake Kantelai, the last named being shallow throughout.†

The algal flora is well developed in most of the tanks, and, as far as the present state of affairs is concerned, their conditions of life are practically natural. The special conditions may be briefly considered first. The illumination is, of course, very intense, for the tanks are, as a rule, exposed to the full glare of the sunlight.‡ Trees and bushes only rarely come down to the water's edge, and the immediate neighbourhood of the bank tends to be inhabited either by dead trees, whose bleached trunks and branches look like spectres in the distance, or, at the best, only by a stunted growth, owing to the frequent changes of water-level. The edges of the tanks on the water-side are generally occupied by a rich growth of water-weeds

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* The termination *wewa* is the Singhalese name for tank, *kulam* is the Tamil designation.

† It is almost impossible to obtain a boat on any of the tanks, and the presence of crocodiles generally makes wading impossible. On the whole, therefore, my observations are confined to the vegetation within a few feet of the water's edge. It would have been interesting to examine a few of the smaller tanks a little more completely, but the littoral region is, after all, generally the most abundantly populated, and most likely to afford important results from our point of view. In all cases I made a point of walking round more than half the embankment of each tank, so as to obtain a good general idea of the type of growth it contained. I was able to investigate Lakes Mineri (by the courtesy of Mr. McPhail) and Kantelai more completely with the help of a boat, and also the lakes at Colombo (by the kindness of the Colombo Rowing Club), and Kandy (by the courtesy of Mr. Baird).

‡ Oltmanns has recently pointed out ('Morph. u. Biol. d. Algen,' vol. 2, 1905, p. 190), that an important consideration with regard to the conditions of illumination influencing aquatic algal growth is the quantity of light which gets reflected at the surface of the water. Not only is the intensity of the light much greater in the tropics, but, owing to the more or less vertical position of the sun, a far smaller percentage of the light is reflected at the surface. I was only rarely inconvenienced by the glare of light from the surface of the tanks (*cf.* also Ewart, in 'Annals of Botany,' vol. 12, 1898, p. 379).

(*Myriophyllum indicum*, *Trapa bispinosa*, *Nymphœas*, *Neptunia oleracea*, *Aponogeton monostachyon*, *Limnophytum obtusifolium*, *Chara*, etc.), and beneath these numerous algal forms generally find shelter. In some tanks the muddiness of the water, in others an abundant Plankton (notably *Clathrocystis !*) serve as a screen to the remaining life in the water. As a general rule (which is, however, by no means without exceptions, *cf.* table on p. 224), a tank with a poor Plankton is not rich in any kind of algal growth (*e.g.*, Kurunegalawewa, Nalandewewa), whilst one with a fairly abundant Plankton generally contains a good deal of other algal vegetation (*e.g.*, Kelawewa, Lakes Mineri and Kantelai). On the other hand, it seems that, in some cases, the Plankton may become so extremely abundant that it does not allow of the passage of sufficient light for any other noticeable growth; this was the case in the tanks at Dambulla (*Clathrocystis !*) and Habarane (*Clathrocystis* and *Pediastrum !*). The Plankton is, of course, in no way the only source of shading in the tanks, and where there is a well-marked growth of water-weeds, for example, these may shelter abundant Algæ, although the Plankton is poor.* The light to which the aquatic Algæ of the tropics are subjected, however, always remains very strong, and it is, therefore, not surprising to find the Cyanophyceæ again predominating (probably owing to their protective pigment, *cf.* p. 206), although, perhaps, not to such a marked extent as in the subaërial vegetation

We have already, in the preceding section, learnt to associate the Cyanophyceous element with a high temperature, and this is, of course, the second condition to which the flora of tropical waters is subjected. Leaving the lakes at Kandy and Nuwara Eliya out of consideration, the approximate mean daily temperature of the water near the edge of the tanks is 28°·5 C.; the lowest temperature observed (6 a.m.) was 25° C., which is only attained on hot summer days in our parts; the highest temperatures (from 10 a.m. to 2 p.m.) range from 30° to 35° C.,† the latter in shallow tanks like most of the smaller ones, as well as Kantelai and Sigiri; in the deeper tanks (*e.g.*, Villamkulam, Dambulla tank) the temperature does not seem to rise much above 30° C. In the shallow tanks the range of daily temperature is very considerable (viz., from 25° to 34°·5 C. in Lake Kantelai, for example) and, in the small tanks, such a wide range is probably the rule. Even in the deeper tanks the range is apparently considerable, and not far behind that of the air (*e.g.*, in Villamkulam, water from 25° to 30° C.; air from 24° to 30°·5 C. in the shade) at least, as far as the surface-layers of the water are concerned. These observations, which, of course, refer to the temperature round the margins of the tanks only, and apply also to all the different kinds of inland fresh-waters, show that a very high temperature is frequently attained during the day, and that there is a considerable, though gradual, cooling over night, which, however, never results in a temperature which would be considered anything but warm in our parts. It seems probable from what is known that a considerable number of fresh-water Algæ prefer rather cold water,* and this may be one of the causes of the practical absence of a number of green forms from the fresh-waters of the tropics (*cf.* below). On the other hand, most of the Cyanophyceæ seem to flourish best in warm waters, and hence their preponderance in the aquatic flora of Ceylon. The relatively high temperature of tropical waters, however, also involves the solution of a proportionally smaller amount of oxygen and carbon dioxide. Especially the smaller percentage of oxygen, and the consequent greater difficulty of respiration, seems likely to be an important feature, although it remains to determine its influence by exact experiment. I incline to asume that this is one of the chief causes of the practical absence of certain temperate genera from the waters of the tropics, and that it also accounts for certain peculiarities of tropical fresh-water algal growth.†

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* Attention may be drawn to the fact that in the absence of water-weeds, the green macrophytic algal growth of the tanks is almost invariably attached; floating green patches only occur in those tanks in which the abundant growth of large aquatics furnishes a considerable shelter against the strong light.

† The temperature of a warm bath is about 35° C.

The temperatures of the upland waters are, of course, in no way so high; thus lake at Kandy (10.30 a.m.) = 25° C.; lake at Nuwara Eliya (8 a.m.) = 16° C., the latter being almost temperate, although on hot sunny days the water probably becomes rather warmer than it ever does in our parts. The range of daily temperature is certain to be very considerable here (*cf.* introduction).

The third condition to which the algal vegetation of the tanks is subjected is a frequent change of water-level, which probably takes place with a rapidity unknown in our parts. During the dry period some of the smaller tanks decrease to more than half their normal size, and are then surrounded by very poor meadow-land with stunted grassy growth, the external portions of which are often occupied by the dead trees above referred to; the limit of the tank in the wet season is well marked by the fringe of dense jungle, which begins some little way behind high-water mark. Towards the end of the dry season even the larger shallow tanks are very much reduced, being surrounded by large areas of meadow-land, marshy in character near the water but dry and bleached beyond. In the deeper tanks (*e.g.*, Mineri, Nuwarawewa) the sinking of the water is not so noticeable, although the bleached algal remains on the exposed stonework of the dams bear testimony to the height of the water in the wet season. The rise of the water-level can take place very rapidly, and the change produced by a single night's heavy rain is remarkable.* As soon as the rains commence the shallower tanks are surrounded by a wide stretch of shallow water devoid of any algal growth; this latter will, of course, put in an appearance subsequently, but the few tanks that I was compelled to examine in the (beginning of the rainy) season gave quite barren results in this respect. That an abundant growth of Algæ and other water-weeds does often take place in the later portion of the rainy period is evidenced by the decaying remains left behind on the sinking of the water-level in the dry season. Where the irrigation-works are in working order they tend to prevent the water-level from rising above a certain height.

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* *Cf.* Oltmanns, *loc. cit.*, vol. 2, 1905, pp. 186 and 187.

† *Cf.* Warming, 'Ökologische Pflanzengeographie,' Germ. Ed., Berlin, 1896, p. 121. "Dass das Absorptionsvermögen des Wassers für Gase mit steigender Temperatur abnimmt, ist vielleicht der wesentlichste Grund, weshalb gewisse Wasserpflanzen im Sommer . . . verschwinden"; *cf.* also *loc. cit.*, p. 124.

More important than the actual variation in the height of the water-level are the consequent sudden changes in the concentration of the matter dissolved in the water; this cannot be without effect on a group so delicately constituted as that of the fresh-water Algæ, and possibly this is a cause for the poor development of the algal flora in some of the tanks.

Whereas the three preceding conditions (light, temperature, and change of water-level) are practically the same in every piece of lowland fresh-water (especially the last two), we have now to consider those factors which differ from tank to tank and are in all probability frequently the cause of the minor variations in the algal flora; these are: chemical composition of the dissolved matter in the water, degree of movement in the water, nature of the substratum, and muddiness of the water. These (together with the conditions of illumination) are just the same features as are probably the main cause of the variation in the algal flora of temperate waters, but in the tropics the dominant conditions (light, temperature) are the determining factors for the general character of the fresh-water flora and, consequently, the minor conditions have a rather different basis to work upon. My time was too limited to enable me to collect many data regarding these minor factors and their bearing upon algal vegetation in the waters of Ceylon, but I saw enough to satisfy myself that a careful study would probably reveal somewhat analogous algal associations in the tropics to those occurring in our parts of the world.

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* According to Mr. McPhail, the local engineer, the water-level in the huge expanse of Lake Mineri may rise as much as 10 feet in the course of one night. This tank is supplied by a number of mountain streams, which, when the rainy season commences, pour large masses of water into it. At Lake Kantelai I observed a rise of several inches in the course of a single night.

I will now briefly consider the minor factors, and will begin with the little I can say regarding the chemical composition of the water. Some of the tanks must certainly be rich in dissolved organic substance owing to the large amounts of water-weeds they contain;* in other cases the fæces of the abundant water-fowl must contribute to the organic contents. The frequent presence of Characeæ probably means dissolved carbonate of lime.

Most of the smaller tanks are sheltered from the ordinary winds, and the water, even at the edge, was usually quite undisturbed at the time of my visit. In the larger tanks, on the other hand, a relatively large surface is exposed to the wind, so that there are usually numerous small waves at the edge; the littoral flora is therefore growing in water, which is frequently disturbed and well aerated (especially noticeable in Nuwarawewa, Tissawewa, and Lake Mineri).

The substratum in the tanks is muddy or clayey except where the tank occupies an excavation in the rock (*cf.* p. 219). In Balaluwewa and Villamkulam part of the substratum seems to be sandy. The dams are often mere clay embankments, but in a number of the larger tanks are made up of blocks of rock, and these rocks, of course, afford a suitable base for lithophytes when under water.

In many of the smaller tanks the suspended particles of mud are of an exceedingly finely divided nature, giving the water a yellow colouration; these particles are so fine that they often take several days to settle completely. The water of these tanks (*e.g.*, Punchikekirawa, Mancadawawewa) is generally, but not always (*e.g.*, tank between milestones 101 and 102 on road from Vavoniya to Madawachya), poor in organic life, and this latter consists mainly of animals.

Briefly summarised, the conditions of life of the macrophytic Algæ in the Ceylon tanks are thus as follows:—Strong illumination; high temperature of considerable daily range (varying from 6° to 10° C.); small amount of dissolved oxygen in the water; periodic (often rapid) change of water-level, causing exposure for the attached forms of the higher zones and change in concentration of the water for the forms of the lower zones; mostly a muddy or clayey substratum with occasional rocks (detached from the dams); water which is generally almost stagnant.

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* Thus, especially in Tank Basawakkulam and Lake Kantelai, the edge was fringed by an accumulation of partly bleached and partly putrid remains of *Chara*, *Myriophyllum*, etc., many yards in extent, and I have little doubt that such a mass of decomposing water-weeds forms the bottom of the entire lake. Round about the edge of Lake Mineri I also found *Chara* washed up in large amount. It is a well-known fact that especially Cyanophyceæ prosper very well in a medium containing dissolved organic substance (*cf.* p. 227, and also Kirchner, in Engler u. Prantl, 'Die Natürl. Pflanzenfam.,' I Teil., Abt. 1*a*, 1900, p. 63).

Looking upon the algal flora of the tanks in its entirety (we may even for a moment include the Plankton in these remarks), one is struck by the fact that in a considerable number of them it is completely dominated by the blue-green element.* The table below may serve as an illustration of this point; the various tanks studied are classified in three series according to the composition of Benthos and Plankton. Those tanks of the first series which are provided with an asterisk contain practically nothing but Cyanophyceæ; the remainder have a noticeable green element, although still entirely dominated by the blue-green. It also seems of interest to notice the relative amounts of Phanerogamic vegetation present;

Table to show Essential Composition of Algal Growth in Ceylon Tanks.

Littoral growth, blue-green. Plankton, blue-green.	Plankton only, blue-green. Littoral growth, green.	Plankton, green. Littoral growth, green.
Colombo Lake (!). **Kandy Lake** (!). **Nuwarawewa.** Interconnected. **Tissawewa** (!). **Basawakkulam.** ***Tank in Anuradhapoora Gardens.** ***Town tanks, Anuradhapoora.** Madokotaikulam (near Vavoniya). **Madawachyawewa.** ***Dambullawewa** (!) (Plankton only!). *Neravieawewa (!). **Habaranewewa** (!) (Plankton only!). Tank at Sigiri. ***Pokuna on top of rock Sigiri** (!) (Plankton only!). **Lake Mineri.** ***Lake Kantelai.** ***Andankulam.** Hiriwadunnawewa (near Habarane).	**Villamkulam.** Tirappanewewa.† **Balaluwewa.** Periyakulam (near Trincomalie).	Pond in Peradeniya Gardens. Interconnected. **Kelawewa.**‡ Mancadawewa† (animals common). Punchikekirawa.‡ Ekiniyeawewa.† Malawewewa.† **Balankulam**‡ (near Anuradhapoora). Madakanawewa.‡ Mahakadawellawewa. Namoluwewa. Megaswewa (!)† (Plankton only!). Wendrenkulam† (Plankton only!). Kurunegalawewa (Plankton only!). Senadiniyagawawewa (!) (near Habarane) (Plankton only!). Borlasgamawewa.‡ Tibbotuwawewewa. Koilpuliumkulam (near Vavoniya). Malawewewa.‡
	Littoral growth only, blue-green. Plankton, green.	
	Nalandewewa (!)†. Walikulam (near Kekirawa). **Karambawewewa.** Yaka-anaguhuwewa (near Habarane). **Mahakekirawa.** Kekunadurewewa (!)†.	

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* I, of course, exclude the green Phanerogamic vegetation, which is often abundantly developed in tanks which are otherwise completely dominated by the blue-green forms.

† Plant life very poorly represented.

‡ With a noticeable admixture of blue-green forms.

where this latter is poorly developed, an exclamation mark in brackets is added.

At first sight this table may not appear to bear out the above statement of the dominance of the blue-green element very clearly, but a consultation of the map will show that all the large and important tanks and lakes come into the first or second columns, the only noticeable exceptions being Kelawewa and Kurunegalawewa.* I may also point out that of the tanks enumerated in the third column, five (all near Kekirawa) are probably in connection with one another and with the waters of Kelawewa, and that a considerable number of them (five) are very poor in organic life altogether, which cannot be said of any of those in the first column. I have endeavoured to bring out this latter point more clearly by employing heavy type for those tanks which have an abundant algal vegetation (either littoral or pelagic or both). It will be seen that whereas nearly all the tanks with a dominant blue-green vegetation are rich in Algæ, this is only the case with respect to two of the tanks in the third column. In further illustration of this point, I append lists of tanks in which the green and blue-green forms respectively are negligible (the same arrangement of type is preserved):—

Green Element negligible.

Basawakkulam.
Tank in Anuradhapoora Gardens.
Town Tanks, Anuradhapoora.
Dambullawewa (Plankton only !).
Madawachyawewa.
Neravieawewa.
Pokuna on top of Sigiri (Plankton only !).
Lake Kantelai.
Andankulam.
Hiriwadunnawewa.

Blue-green Element negligible.

Mancadawawewa.
Punchikekirawa.
Tibbotuwawewewa.
Malawewa.
Senadiniyagawawewa (Plankton only !).
Namoluwewa.
Megaswewa (Plankton only !).
Wendrenkulam (Plankton only !).
Kurunegalawewa.
Borlasgamawewa.
Mahakadawellawewa.

As an outcome of these considerations, we may state that whenever there is a well-developed algal flora in the considerable number of Ceylon tanks and lakes I have examined there is *always a noticeable blue-green element which very frequently dominates the entire algal growth.* If we now attempt a comparison of the features just discussed with those of other tropical regions, we meet with serious difficulties, for our existing tropical algal floras, though doubtless of considerable systematic and phytogeographical value, are practically useless from this point of view. I have nowhere found a particular comment on the dominance of the blue-green element, although Messrs. West and West* remark on the presence of well-developed individuals of *Clathrocystis* in tropical Plankton. However, I do not hesitate to suggest that in other parts of the tropics the blue-green element plays the same important part that it does in Ceylon, in some cases to a less, in others, perhaps, even to a more marked extent, according as the external conditions vary. I shall return to this subject elsewhere,† and need here only point out that in view of the very distinct aspect of the fresh-water algal vegetation in the tropics it is certainly astonishing that its general character has not previously been subjected to a critical analysis.

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* It is an interesting point that the dominance of the blue-green element is most pronounced in the larger tanks. The smaller tanks probably present conditions somewhat analogous to those prevailing in the smaller pools and ditches (viz., greater risk of sudden desiccation, and stagnant water with less aeration ; *cf.* the next section of this chapter). The smaller tanks thus gradually carry us over to the small pieces of water considered subsequently.

I will now endeavour to give a brief sketch of the essential character of the blue-green macrophytic Algæ (excl. Plankton), and of their mode of occurrence in the different tanks of Ceylon.‡ The blue-green vegetation forms either (*a*) a dense felt or tufted growth or adhesive film on submerged rocks, tree-stumps, etc.; or (*b*) floating masses, the latter in particular often attaining a very prominent development. The more important and characteristic floating forms belong to the genera *Oscillaria*, *Lyngbya*, *Tolypothrix*, *Scytonema*, *Hapalosiphon*, and *Rivularia*. A member of the second genus (*L. majuscula* (Dillw.) Harv.) is exceedingly common in many of the tanks (*e.g.*, Nalandewewa, Tissawewa, Balankulam, Madawachyawewa, Habaranewewa, etc.), forming large dark-green floating masses of densely interwoven coarse filaments. At Nalande and Anuradhapoora it completely dominates the vegetation of all the waters fed by the respective tanks; at the latter place many of the smaller tanks threaten to become completely silted up by the growth of this Alga and by the mud which collects amid the tangle of its filaments. Other (smaller) species of *Lyngbya* also occur in the tanks (*e.g.*, in Villamkulam, Andankulam), but play no very important part (see, however, the next page).

Another very important constituent of the algal vegetation of the tanks are species of *Rivularia* (*Glœotrichia*). They absolutely dominate the algal flora in Basawakkulam (*Glœotrichia Rabenhorstii*, Bornet), and also play a great part

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* West and West, "Observations on the Conjugatæ," 'Annals of Botany,' vol. 12, No. LXV, 1898, p. 36.

† 'Annals of Botany,' April, 1907, p. 243 *et seq.*

‡ When the water of the tanks falls in the dry season, a number of pools are usually left behind, which, of course, again constitute a part of the tank in the wet season. The vegetation of these pools is taken into account as a constituent part of that of the tank in the following remarks, wherever it is deemed necessary.

in Lake Kantelai (*Glœotrichia natans* (Hedw.), Rabenh.). These two tanks, it is interesting to notice, are both characterised by a very rich growth of water-weeds, so that their waters certainly contain a very considerable percentage of organic substance (*cf.* above, p. 223, footnote); further, they are both practically stagnant. Whether these factors are directly related to the abundant occurrence of *Rivularia* in these tanks must for the present remain an open question. Whereas in Basawakkulam the *Glœotrichia* is the only common Alga, this cannot be said of Lake Kantelai, where another blue-green form (*Lyngbya œrugineo-cœrulea* (Kütz), Gom.) is exceedingly common, and shares the dominance of the former. The *Lyngbya* (like the *Glœotrichia*) is attached to the segments of the abundant water-weeds, covering them with its thin flocculent films for considerable stretches: in places this growth is so profuse that it forms thick yellowish-green masses immediately below the surface of the water. Exactly the same species occurs in abundance attached to the submerged aquatics (*Myriophyllum* and *Chara*) in the open waters of Lake Mineri (together with small quantities of *Glœotrichia*), but here it forms very delicate films, tending to break up on being touched. In both Mineri and Kantelai, however, the films do not consist of pure *Lyngbya*, but give shelter to a considerable number of unicellular forms (various blue-green species; Diatoms, notably *Epithemia* and *Pinnularia;* Desmids, *e.g.*, species of *Cosmarium*), the whole constituting a very characteristic assemblage of algal species. The Diatoms and Desmids occurring in these films are in part quite identical for the two tanks, and the films are thus a good example of the "consortia" discussed below.* It is only open to surmise as to whether there is anything more than a kind of "space-parasitism" in the case of these composite films,† but there seem possibilities of more important (nutritive ?) interrelations which we are not yet in a position to understand.

Oscillaria, though rarely of great importance in the floating flora of the tanks, was a prominent feature in Mahakekirawa; here *O. tenuis*, Agardh. var. *natans* (Kütz), Gom. occurred as small floating discs (2 to 6 inches in diameter, and often almost perfectly round) on the surface of the water. The filaments are arranged in a radiate manner in these discs, which also include a considerable quantity of yellowish mud, serving to increase their compactness. No doubt the discs originate on the mud at the bottom of the tank, and are ultimately raised to the surface by gas-bubble development (*cf.* below, p. 240). Similar discs are sometimes to be observed in temperate regions, but their development has, as far as I am aware, not been studied.

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* See p. 247 and *cf.* Fritsch, "Problems in Aquatic Biology," etc., 'New Phytologist,' vol. 5, 1906, p. 157.

† Inasmuch as Lakes Mineri and Kantelai are the only large tanks I have had the opportunity of studying from a boat, I consider it very probable that such *Lyngbya*-films may occur in the open water of a number of the other tanks, especially as the films are not at all commonly found near the banks, but attain their greatest development over the deeper portions of the tank.

Scytonema (*S. tolypotrichoides*, Kütz) was a dominant form in only two of the tanks, viz., Madokotaikulam near Vavoniya and Karambewawewa, near Anuradhapoora. In both cases it formed spherical ægagropilous masses floating round the margin and consisting of large quantities of knotted, more or less radially arranged filaments, frequently of a yellowish colour on the surface, although deep blue-green in the interior of the spherical structure. The tangle of filaments, no doubt, includes air, by virtue of which the masses, which are often about double the size of a cherry, float. The mode of development of these masses has not been studied, but is probably analogous to that of the ægagropilous *Cladophoras*,* depending on successive movement of the growing structure into various positions by the currents in the water.

There is much greater specific diversity to be found in the attached Cyanophyceous growth than among the floating forms, which, as the above remarks will have shown, are referable to a small number of species; the most important attached forms belong to the genera *Oscillaria*, *Lyngbya*, and *Tolypothrix*. In comparison to our parts, the practical absence of unicellular species in the attached blue-green flora is noteworthy. The dense pilose tufted growth found on submerged rocks (especially Lake Kantelai), tree-stumps, etc., is usually not due to a single species, but is composite (both blue-green and green with intermingled Diatoms, viz., *Synedra*, *Navicula*, *Surirella*). There is probably no essential difference between the attached growth in the majority of the tanks, and that found in many of the small pools (*cf.* below); in both cases we are dealing with algal consortia.†

We will now turn our attention to the composition of the green algal element in the flora of the tanks. It exhibits a number of characteristic features (common in part to the algal flora of all the inland waters), which are, on the whole, just as striking as the dominance of the Cyanophyceæ, viz.:—

(*a*) The practical absence or great scarcity of certain genera, such as *Cladophora*, *Rhizoclonium*, *Vaucheria*, *Ulothrix*, and *Conferva*, and the consequent scarcity of a number of epiphytic Diatoms so characteristic of the freshwater algal flora of our parts (*e.g.*, *Cocconeis Placentula*, *Synedra splendens*).

(*b*) The replacement of *Cladophora* by the genus *Pithophora*.

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* *Cf.* especially Brand, "Die *Cladophora*-Ægagropilen des Süsswassers," 'Hedwigia,' vol. 41, 1902, p. 34 *et seq.*; and Wesenberg-Lund, "Sur les *Ægagropila Sauteri* du lac de Sorö," 'Bull. Ac. R. d. Sci. et d. Lettres Danemark,' 1903, No. 2, p. 167 *et seq.*

† A few further facts regarding the attached growth on rock-surfaces in the tanks are mentioned in the section dealing with the algal flora of the rock-pools (p. 248).

(*c*) The important part played by the genus *Spirogyra* to the practical exclusion of other filamentous Conjugates, and the special systematic composition of this *Spirogyra*-element.

(*d*) The usually marked absence of broad filamentous forms (excepting *Spirogyra*).

The genus *Cladophora* and its ally *Rhizoclonium* are exceedingly rare in Ceylon,* and the explanation of their absence seems no very difficult one. In our parts they generally frequent water which is well aerated, and in larger pieces of water, for instance, are in great part confined to near the banks, where there is plenty of motion owing to the wind.† The practical absence of these two genera in the fresh-waters of Ceylon is probably due to an insufficiency of dissolved oxygen. The thick coarse walls and broad filaments of these genera are not well suited for a ready diffusion of gases. The absence of *Vaucheria* also may be due to its broad filaments, although (as far as the tanks are concerned) this genus is not a very common constituent of lake-vegetation, even in our parts. *Vaucheria*, however, has a decidedly different assimilatory process to that of most other green Algæ, and it is possible that this process does not go on readily amid tropical conditions. This view may receive some measure of support in the fact that the entire group of the Heterokontæ (Confervales, Borzi), which probably have a similar assimilatory process,‡ is very rare in the lowlands of Ceylon.§ Ulotrichaceæ are certainly also§ rare in Ceylon, and their scarcity is not easy to understand. Some species of *Ulothrix* are known to favour aerated water (although this does not apply to all of them), and possibly the small percentage of dissolved oxygen may have something to do with their absence. We have practically no data regarding the influence of the amount of dissolved oxygen on the different genera of Algæ, but it seems very likely that some of them may be far more susceptible in this respect than we imagine, so that tropical conditions may even exclude forms which favour stagnant water in our parts.

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* The following remarks (to last paragraph on p. 231) apply to the fresh-waters of the Ceylon lowlands as a whole.

† *Cf.* Chodat, "Algues vertes de la Suisse," 'Pleurococcoïdes—Chroolépoïdes,' Berne, 1902, p. 77; also p. 90.

‡ Regarding this assimilatory process, see especially K. Bohlin, "Studier öfver några slägten af Alggruppen Confervales, Borzi," 'Bih. K. Svenska Vet.-Ak. Handl.," vol. 23, Afd. III, No. 23, German Résumé, pp. 52—54. The chloroplasts contain a relatively large amount of xanthophyll, and the first product of assimilation appears to be a glucose, which subsequently becomes transformed into oil. The assimilation is thus decidedly different to that of the remaining green Algæ, and in the conditions of illumination, temperature, etc., prevalent in the tropics, there may be some factor or factors unfavourable to the process of photosynthesis in this case.

§ Messrs. West and West ("Fresh-water Algæ of Ceylon," 'Linn. Soc. Trans.,' vol. 6, 1902, p. 124) have already commented on this subject.

We will now briefly notice the occurrence of representatives of the groups and genera just discussed in Ceylon waters. I have practically only met with fresh-water species of *Cladophora* in two wells (at Matale and Ambalangodda, see p. 250), *i.e.*, in well aerated water. *Rhizoclonium*, a genus which is exceedingly common in our temperate waters, was scarcely met with except in the estuaries and lagoons. On the other hand, the most important representative of the Cladophoraceæ in Ceylon is quite undoubtedly the genus *Pithophora*, which, although neither a very common form, was met with sufficiently frequently to make it a sensible constituent of the algal flora. It was never found in the actual tanks except in those of small size, but generally occurred in the side-pools above mentioned (p. 226, footnote). The species of *Pithophora* have, on the whole, thinner cell walls* than either *Cladophora* or *Rhizoclonium*, so that they may be in a better position to withstand the increased difficulty of respiration in the tropics. *Pithophora* may, however, in its spores also have a far better adaptation to the vicissitudes of the tropics (desiccation) than either of the two other genera;† this is a point which should not be very difficult to settle experimentally. If we examine into the existing records of the occurrence of species of *Cladophora* and *Rhizoclonium* in the tropics, we find that in most cases (where the habitat is at all adequately described!) these forms were growing in flowing or otherwise well aerated water.‡ The remaining cases do not prove anything to the contrary until it has been definitely ascertained that the water in which these forms were growing was actually stagnant. I think it may be safely stated that both *Cladophora* and *Rhizoclonium* are rare forms in tropical fresh-waters, and that they normally only appear in a well aerated habitat. They are replaced by the genus *Pithophora*, which apparently flourishes quite well in stagnant waters.

I have met with *no species* of the genus *Vaucheria* in the lowlands of Ceylon (terrestrial and aquatic species in the uplands! *cf.* pp. 217, 254), although one may regard my collections as fairly representative of the algal flora. A consideration of the published papers further brings out the startling fact that very few determinable fresh-water species of this genus have been recorded from the tropics, and there are only few records of terrestrial species (chiefly *V. sessilis* (Vauch.), DC.).* In the course of my travels I came across many likely localities for the occurrence of *Botrydium*, but I never found it; there are only three records from the tropics (from Loanda in Africa, Brazil, and Ecuador). We may, therefore, further characterise the algal flora of the tropics by the practical *absence of fresh-water species of Vaucheria*, and the extreme scarcity of the terrestrial Siphoneæ (*Vaucheria* and *Botrydium*).

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* See especially Wittrock, "On the Development and Systematic Arrangement of the Pithophoraceæ," 'Nov. Act. Reg. Soc. Sc. Upsala,' Ser. 3, 1877, p. 8: "The vegetative cells . . . . have all a thin membrane of cellulose without layers. In Cladophoreæ, particularly the cells belonging to the lower part of the thallus have often a thick membrane in distinct layers." See also the plates illustrating the paper cited.

† It is interesting in this connection to mention that Miss F. Rich, working jointly with me on the biology and ecology of British fresh-water algal growth, has come to the conclusion that *Cladophora* deserts those ponds which are subject to drying up in summer.

‡ The records of tropical Algæ found in the existing literature will be considered more fully elsewhere ('Annals of Botany,' vol. 21, No. LXXXII, April, 1907, p. 248 *et seq.*).

I must here refer to another feature which is intimately connected with the rarity of *Cladophora*, *Rhizoclonium*, and *Vaucheria*, viz., the scarcity and different composition of the epiphytic growth, which in our waters finds one of its main substrata on the filaments of these genera. The characteristic epiphytic Diatoms (*Cocconeis*, species of *Gomphonema*, *Synedra splendens*, etc.) are very rare in the Ceylon fresh-waters, which may, in part, be the result of the lack of a suitable substratum. But even where I found *Pithophora* with a well-marked epiphytic growth, it was not made up of the above-mentioned species of Diatoms, but usually consisted largely of species of *Achnanthes*.

The entire group of the Confervales seems to be poorly represented in the tropics; only three genera (*Characiopsis*, *Conferva*, and *Ophiocytium*) have been recorded and, except in the case of *Ophiocytium*, the records are rather few in number.† In Ceylon I have only met with occasional filaments of *Conferva* in fresh-water, although future more detailed examination of my material may disclose small numbers of the unicellular forms. Whether the peculiar type of assimilation in this group is really responsible for its scarcity in the tropics can only be settled by a careful investigation of those tropical localities in which members of this group appear.

The Conjugates are amongst the most important constituents of the green element in the algal flora of the tanks, and it will be best to consider the Conjugate vegetation of these latter separately from that of the other pieces of Ceylon fresh-water. The Conjugates are best developed in those tanks which possess a rich growth of water-weeds; where larger aquatics are wanting, conditions are not favourable for the growth of any floating green Alga, which is bound to rise to the surface during the hot hours of the day, and so be fully exposed to the strong sunlight. The development of the filamentous Conjugate element in the tanks takes place almost entirely at the expense of species of *Spirogyra*; *Mougeotia* is exceedingly rare (*M. parvula*, Hass., var. *angusta*, Hass. in Tissawewa), whilst I never met with a *Zygnema* in any of the tanks. Looking at the species of *Spirogyra* found in the Ceylon freshwaters in their entirety, it is very noticeable that they all belong to the section in which the transverse walls between the cells are simple and not infolded, and that the large majority have more than one or even two spiral chromatophores in each cell. These features are, in the main, common to all the *Spirogyras* of tropical waters, although they do not appear to have been noted before. Of 38 species recorded from the tropics, only nine* have cells containing a single chloroplast, and only five have infolded transverse walls. Infolding of the end-walls of the cells is commonly met with in many of the narrower *Spirogyras* of our waters, and is generally regarded† as a mechanism for the dissociation of the cells of the filament, but its exact function is not perfectly understood, nor do we know why it occurs in one species and not in another. Since the large majority of tropical *Spirogyras* are broad forms, and infolded walls are only found in the narrower species, we could in no case expect to find many examples of the latter type; yet the interesting fact remains that the few narrow forms nearly all have simple end-walls. It is just as difficult to account for the prevalence of broad forms with numerous closely-arranged chloroplasts in each cell. Except for the species of *Pithophora*, the *Spirogyras* are the only Algæ with broad filaments found at all commonly in the tropics, all the remaining forms being narrow and rarely exceeding 15 μ in diameter. I am inclined to associate this with the fact that *Spirogyra* gets on well in stagnant water under relatively unfavourable conditions of respiration (perhaps owing to the thin cell-wall), so that forms with broad filaments are as successful as the narrow ones (*cf.* next paragraph and also p. 221). I am unable to offer any explanation for the usual abundance of chloroplasts.

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* *Cf.* 'Annals of Botany,' *loc. cit.*, p. 254.

† *Loc. cit.*, pp. 256, 257.

The most important forms in the attached green algal flora are species of *Œdogonium*.‡ Floating masses of this genus are rather rare (only in Madokotaikulam and Madakanawewa), although this may be a seasonal phenomenon. The species of *Œdogonium* found in the waters of Ceylon are, as a rule, narrow forms with filaments having a diameter of 9—12 μ, even 15 μ being rather rare; species with broad filaments are exceptionally rare. The numerous records of species of *Œdogonium* from the tropics show a very considerable preponderance of narrow forms.* As already pointed out above, the filamentous algal flora of the tropics is prevalently narrow, *Spirogyra* and *Pithophora* being the only broad forms at all commonly met with. This is no doubt due to the smaller percentage of dissolved oxygen in the water, forms with narrow filaments being better suited to these conditions than broader ones. The phenomenon finds its parallel in the extensive subdivision of the foliage in many of the higher aquatics. *Spirogyra* and *Pithophora* are probably able to exist as broader forms, owing to their thin cell-walls admitting of more rapid diffusion of gases. A careful study of the tropical habitats of broad species of *Œdogonium* will probably disclose some special factor favouring their development. Possibly the rather limited occurrence of *Pithophora* may also be due to the necessity of some special condition. The *Spirogyras*, however, are too widely distributed to admit of such an assumption.

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* Sixty-two records of *Spirogyras*, with a number of chloroplasts, as compared with 20 records of forms with a single chloroplast. The details will be given elsewhere (Fritsch, in 'Annals of Botany,' vol. 21, No. LXXXII, April, 1907, p. 260 *et seq.*).

† *Cf.* Oltmanns, *loc. cit.*, vol. 1, 1904, pp. 57, 58. On the whole, my material shows very few cases of breaking up of the filaments into their constituent cells, though the conditions must frequently have been favourable for dissociation. This is probably due to the absence of those species of *Spirogyra* (viz., those with infolded end-walls), which most commonly exhibit this phenomena.

‡ The genus *Bulbochæte* is of rare occurrence in the tanks, and was always only observed in small quantity amongst other attached Algæ.

The Chætophoraceæ do not constitute a very important element, but species of *Stigeoclonium* were observed in some amount in a few cases (*e.g.*, Balaluwewa, Habaranewewa), whilst *Aphanochæte* is occasionally found as an epiphyte on other filamentous Algæ. *Chætophora* was not seen in the tanks. *Coleochæte* is an occasional epiphyte.

The lake at Nuwara Eliya, the only large piece of upland water studied, could, unfortunately, only be subjected to a very cursory examination. There is no doubt, however, that the flora is in no way dominated by the blue-green element. At the time of my visit there was very little algal growth.

(ii) *Algæ of Roadside Ditches and Pools in the Lowlands* (excluding rock-pools, see Section iv).—Although the tanks are the most important pieces of inland fresh-water in Ceylon, a considerable bulk of algal growth is also found in the numerous small pools and roadside drains occurring all over the island. The general character of this vegetation is in most respects similar to that of the tanks, but there are certain noteworthy differences which require a brief discussion. The above-mentioned pools (p. 226, footnote) which are found round about the tanks in the dry season, but constitute part of them when the water rises, occupy an intermediate position between isolated pools and the actual tanks as far as their algal flora is concerned. They generally, however, approach more nearly to the latter, since their flora is (to a great extent at least) dependent on that of the tanks, and is consequently dominated by rather different conditions than apply to the pools, etc., which form the chief subject of discussion in the present section. Most of the forms found in the pools around the tanks are also met with in the main waters of the latter, but from what I have seen I think it probable that certain species are able to flourish better in small isolated pieces of water than in the large expanse of the waters of the tanks, and consequently attain to a degree of development in these pools during the dry season, which is impossible except in such small collections of water. Further, algal spores carried by the wind or other means of dispersal may produce an algal growth in the side-pools, which would never be successful in the main tank.

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* *Cf.* Fritsch, 'Annals of Botany,' April, 1907, p. 265 *et seq.*

The conditions to which the Algæ of the pools and ditches are exposed are the same as those above defined for the tanks, although most of them are more emphasised.* Thus, except where shading is afforded by the surrounding trees and bushes, the shallow character of these pieces of water means little or no protection from the strong light, and in the absence of shade algal growth is rarely found. The temperatures attained are very high, and there is a very considerable daily range. The risk of desiccation is great, and drying up or inundation can take place very rapidly. The water is in almost all cases quite stagnant, heavy downpours of rain being the only source of aeration. The conditions of life are on the whole more uniform than in the tanks, the most essential variations lying in the degree of shading and the frequency of desiccation, conditions which are to some extent mutually dependent.

In the pieces of water under consideration the blue-green element, although still well developed, does not play the same predominant part as in the tanks. The Conjugates are here the dominant group, almost every pool or ditch examined containing a certain and often very considerable amount of *Spirogyra*,† whilst other Conjugate genera (especially *Zygnema*, more rarely *Mougeotia*) are of frequent occurrence, though rarely present in great quantity. The dominance of *Spirogyra* is very noticeable on comparing the vegetation of these pieces of water with that of the segregated pools round about the tanks, for in the latter *Spirogyra* only occupies the second place, as in the actual waters of the tanks. It is not easy to understand why the Conjugates play so important a part in the pools and ditches, whilst the Cyanophyceæ are subsidiary to them. It seems very probable that it is not so much the occurrence of special factors which favours the Conjugate element, but rather the existence of one or more conditions unsuitable to the blue-green element, so that it becomes weakly developed and gives room for the *Spirogyra*, etc.

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* Some of the smaller shallow tanks come very near to the pieces of water under consideration (*cf.* footnote on p. 225).

† The species of *Spirogyra* in the pools and ditches show the same general features as those of the tanks (viz., simple end-walls and a prevalence of broad forms with many spirals in each cell).

Thus the *Spirogyras* may be better suited to meet the emergencies of desiccation than most of the aquatic Cyanophyceæ (which generally have a rather poorly developed sheath as compared with the subaërial forms) owing to the ease with which they form resting spores. Azygospore-formation is not uncommon in my material, and although we are quite ignorant as to the rapidity with which such azygospores are formed, it seems reasonable to conclude that it is a much more rapid process than the sexual development of zygospores. In case of approaching desiccation, which in the tropics will often take place very rapidly, there will be a sudden concentration of the water, and this may well act as a check on ordinary conjugation and lead to the formation of azygospores.* The prevalence of *Spirogyra* may, however, also be due to the frequent strong shading† of these pieces of water, or to the success of *Spirogyra* in perfectly stagnant waters such as these, both conditions which though, perhaps, not unfavourable to the blue-green element, may still enable the Conjugates to flourish and crowd out the Cyanophyceæ. In comparison with pools and ditches in temperate regions, however, the blue-green Algæ still play a relatively important part, whilst Conjugates are much more strikingly abundant. Without there being much difference in generic constitution the facies is quite another one.

In a number of the pools and ditches examined, *Spirogyra* was practically the only form present, but in other cases it is accompanied by a subsidiary vegetation composed of *Œdogonium, Microspora, Zygnema, Mougeotia, Bulbochæte, Ulothrix, Stigeoclonium,* and various Cyanophyceæ‡ (*Anabæna, Lyngbya, Oscillaria, Scytonema,* etc.); in a few cases one or other of these forms (nearly always *Œdogonium*) was dominant, while the *Spirogyra* takes a second place.

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* *Cf.* Klebs, 'Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen,' Jena, 1896, p. 246 *et seq.* In *Spirogyra Weberi* a 4-per-cent. solution of cane-sugar induced the formation of a large number of azygospores, whereas in *S. inflata* a 6-per-cent. solution was necessary to interfere with ordinary conjugation and produce parthenogenesis; in this latter species a 4-per-cent. solution induces ordinary conjugation, so that a sudden increase in concentration from 4 to 6 per cent. leads to parthenogenesis.

† Messrs. West and West ("Observations on the Conjugatæ," 'Annals of Botany, vol. 12, No. XLV, 1898, pp. 35, 36) point out that the Conjugates can stand prolonged exposure to fairly strong light in their natural habitat, which is not quite in agreement with the statement that the effect of such exposure is to induce the formation of zygospores (*cf.* also Ewart, "The Action of Cold and of Sunlight upon Aquatic Plants," 'Annals of Botany,' vol. 12, 1898, p. 379 *et seq.*). It would seem as though strong light stimulated the reproductive functions which probably means cessation of the vegetative activities. On the other hand, Conjugates appear to be rather less sensitive to illumination than many of the other filamentous green genera, and it is possible that the tropical members of this order are adapted to a fairly strong light.

‡ Except for occasional floating forms, the Cyanophyceæ occur most commonly attached to water-weeds and branches and twigs lying loosely in the water.

It is interesting that if the latter genus is entirely wanting Cyanophyceæ generally take the lead (*cf.* the remarks in the preceding paragraph). Desmids are occasionally very abundant, whilst other unicellular and colonial green forms (Protococcales, etc.) are rare in these lowland waters; colonial Cyanophyceæ (*e.g.*, *Merismopedia*, *Gomphosphæria*) are sometimes not uncommon. In comparison with temperate regions the scarcity of Diatoms is very noticeable, which is not difficult of explanation when one recollects that these forms have a preference for cold water, and even in our parts generally attain their maximum during the winter months. Yet both Diatoms and Desmids are a good deal commoner in the pools and ditches than in the tanks.

The large majority of these pools and ditches of the lowlands are characterised by a *Spirogyra-Œdogonium** vegetation, the latter genus, though relatively rarely attaining abundant development, being almost invariably present in some quantity. This vegetation acquires different aspects according to the subsidiary forms present and is particularly well emphasised in certain pools and ditches owing to the almost constant association of other species with the *Spirogyra* and *Œdogonium*.

The pools and ditches thus characterised always exhibit a dense, brownish-red granular deposit on the bottom, and the same deposit often also constitutes a filmy investment to all the water-plants. This deposit consists of ferric hydroxide, and I will consequently talk of these pieces of water as ferruginous pools.† Such pools are very common in all the lowland regions with a considerable rainfall (*e.g.*, Negombo, Colombo, Hanwella, etc.), and are not rare in the uplands around Nuwara Eliya. Owing to the uniform character of the algal flora, these ferruginous pools may all be considered together (*i.e.*, both those of the lowlands and uplands). In a dozen typical cases examined from different parts of the island, neither *Spirogyra* nor *Œdogonium* were ever found to be absent from the pools, although the one or the other (very rarely both) was occasionally only present in small quantity. Of other filamentous forms, various species of *Bulbochæte* (including *B. minuta*, West and West, *B. spirogranulata*, West and West, etc.) are rarely wanting, and may be regarded as a constituent of almost equal importance to the two genera previously mentioned; they invariably occur as epiphytes on larger water-weeds, and possibly their absence in some few cases is the result of the lack of a suitable substratum. *Ulothrix*,

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* The species of *Œdogonium* found in these pieces of water are again prevalently narrow (see p. 232).

† Similar deposits are also found in the rice-fields, and these waters show the same characteristic flora as that now to be described.

Microspora, and *Zygnema* are all casual forms in these pools, these being the only habitats in which species of *Ulothrix* occur at all commonly in the island. Of much greater importance for the characterisation of the ferruginous pools are certain Desmids (fig. 3) and Diatoms. Foremost among the Desmids are species of *Pleurotænium* (*P. Trabecula* (Ehrenb.), Näg.; *P. annulatum,* West and West; *P. trochiscum,* West and West; *P. nodosum,* Lund.; *P. doliforme,* West and West, etc.); diverse forms of *Triploceras gracile,* Bail.; species of *Closterium* (*C. setaceum,* Ehrenb.; *C. abruptum,* West; *C. Pseudodianæ,* Roy; *C. Venus,* Kütz, etc.; in the uplands, also:

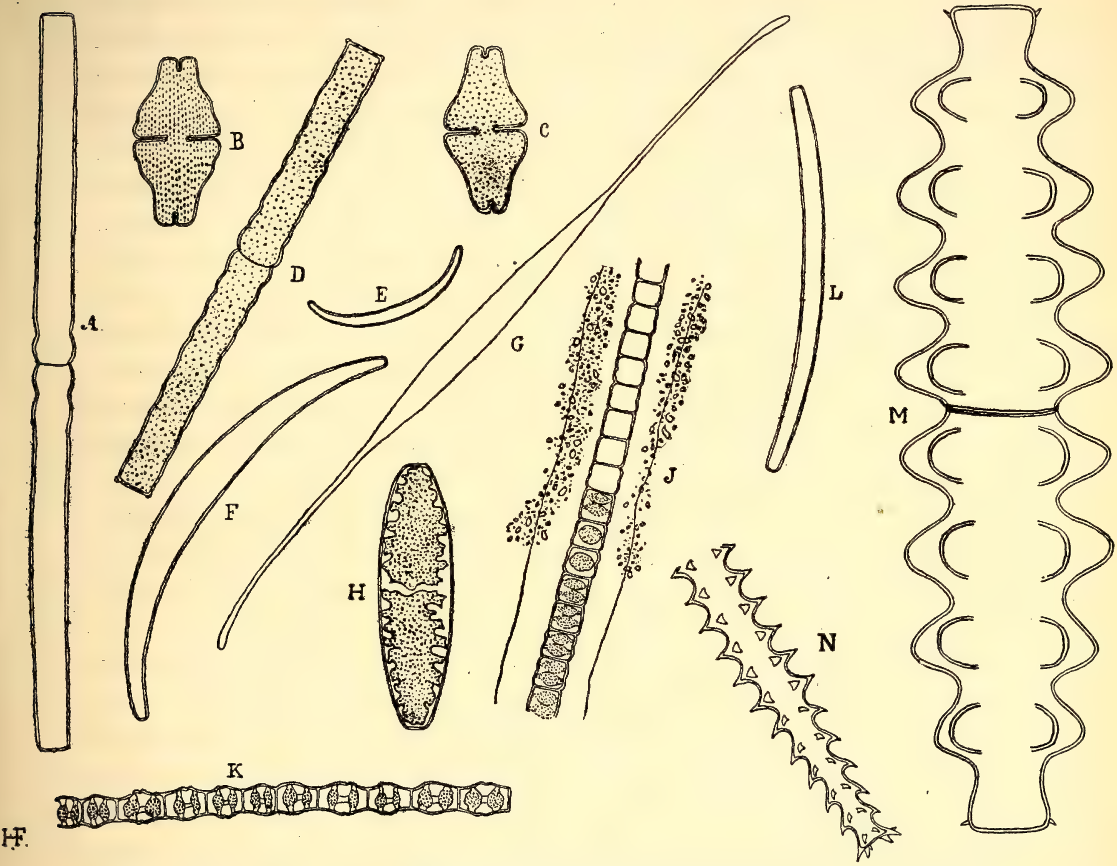

Fig. 3.—A few of the more Characteristic and Typical Desmids of the Ferruginous Pools (all magnified about 260 diameters). A, *Pleurotænium Trabecula* (Ehrenb.), Näg. B, *Euastrum ansatum,* Ralfs. C, *Euastrum obesum,* Joshua f. *punctatum.* D, *Pleurotænium annulatum,* West and West. E, *Closterium Venus,* Kütz forma. F, *C. pseudodianæ,* Roy. G, *C. setaceum,* Ehrenb. H, *Netrium digitus* (Ehrenb.), Itzigs. and Rothe. J, *Hyalotheca dissiliens,* Bréb. K, *Gymnozyga moniliformis,* Ehrenb. L, *Closterium abruptum,* West. M, *Pleurotænium nodosum,* Lund. N, *Triploceras gracile,* Bail.

C. Leibleinii, Kütz, and *C. Jenneri*, Ralfs); species of *Euastrum* (*E. ansatum*, Ralfs; *E. obesum*, Joshua; *E. dideltoides*, West and West); and *Netrium digitus* (Ehrenb.), Itzigs. and Rothe; *Cosmarium*, *Penium*, *Staurastrum* (more frequent in the uplands!) and *Spirotœnia* are of minor importance and often scarcely represented. A number of filamentous Desmids* (*Gymnozyga moniliformis*, Ehrenb.; *Hyalotheca dissiliens*, Bréb.; *Micrasterias foliacea*, Bail.;† and more rarely species of *Desmidium*) are also characteristic forms in these pools. Amongst the Diatoms, *Pinnularia* and various small species of *Navicula* play the most important part, although only of occasional occurrence. *Pleurosigma* and *Surirella* seem to be characteristic in some cases, whilst, where water-weeds are present, *Synedra lunaris*, Ehrenb., sometimes occurs as an epiphyte on them. The blue-green element is often entirely wanting in these pools, and is never of any importance.

Before proceeding to discuss the few data available regarding the biology of this flora, it will be well to briefly sum up its constitution as follows:—

(a) *Dominant.*—Species of *Œdogonium* and *Spirogya*, *Pleurotœnium Trabecula*, *P. annulatum*, *Closterium setaceum*, *C. pseudodianœ*, *Triploceras gracile*, *Netrium digitus*.

(b) *Subsidiary.*—Species of *Bulbochœte*, *Euastrum*, *Pleurotœnium*, *Closterium*, *Cosmarium*, *Pinnularia*, *Navicula*.

(c) *Occasional.*—The remaining forms mentioned above and a considerable number of others.

Of all the forms enumerated, only *Spirogya* and *Œdogonium* are absolutely constant, all the others being occasionally absent.‡ Their absence may be due to actual variations in this ferruginous flora, or to the pools examined being in diverse phases, the latter, on the face of it, seeming the more probable explanation. This brings us to consider the relation between the algal flora and the characteristic granular deposit found in these pieces of water. We are confronted by two possibilities. The flora may have nothing to do with the deposit, or the latter may be due to the activity of the Algæ present. Such evidence as I have been able to obtain points to the latter conclusion. Whenever there is a considerable growth of filamentous Algæ in these pools, large amounts of the granular deposit form a dense, reddish-brown flocculent investment all round the filaments (see, for example, fig. 3, J), so that the latter are often completely obscured; a similar coating is generally also found on any water-weeds present. It is hardly possible to account for this investment in any other way than by the assumption that it has originated round about the algal growth and water-weeds, as a result of their metabolic activities. The water of these pools contains a small amount of dissolved ferric chloride,* and this is probably transformed (oxydised) into an insoluble form by the vital activities of the plants present. As the ferric hydroxide accumulates, it sinks down to the bottom of the pool, and forms a granular layer there.† The gradual increase in the amount of the deposit may produce less favourable conditions (shading ?) for certain algal forms, which thereupon disappear. In the diverse pools studied, many different cases suggestive of the above theory were met with. Thus, in some of the pools, the granular matter merely formed a flocculent covering to the contained vegetation, and there was little or none on the bottom; in others, there was a quantity of the reddish-brown deposit on the bottom, as well as enveloping the plant-growth present; whilst, in still others, there was only a dense granular layer on the bottom, and practically no vegetation rising up into the water above it. In the pools last mentioned the vegetation was generally scanty and least satisfactory, from the point of view of uniformity with the others. Such pools generally also contained a quantity of *Leptothrix*, and it seems as though this genus finds favourable conditions as the deposit accumulates; possibly it is the direct cause of the disappearance of the main mass of algal growth. In any case, the ultimate result of the accumulation of the red deposit is the elimination of a considerable mass of the vegetation. What this process leads to finally I am unable to say, but there are some indications of the initial state of affairs to be found in pools and ditches having no trace of the characteristic deposit, but containing a flora very similar to that above described.

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* Filamentous Desmids are, however, only a marked feature of the lowland pools; they are rarely present in those of the uplands (*cf.* p. 253). There is a very marked filamentous tendency amongst these Desmids of the lowland pools; this is exemplified by the presence of a filamentous species of *Micrasterias*, a genus in which the individuals are usually single, and by the frequent occurrence of short filaments of *Triploceras gracile* and *Pleurotœnium* (*e.g.*, *P. annulatum*).

† This is the only species of *Micrasterias* I have as yet noticed in these pieces of water.

‡ *Pleurotœnium*, *Bulbochœte*, *Closterium setaceum*, *Triploceras*, and the species of *Navicula* are most widely present.

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* I have to thank Professor E. C. C. Baly, of University College, London, for kindly analysing this water.

† It is not impossible that iron-bacteria may have a hand in this process. They are well known to occur in such waters, and by their activity to give rise to extensive deposits of hydroxide of iron (*cf.* S. Winogradsky, "Üb. Eisenbacterien," 'Bot. Zeit.,' vol. 46, 1888, pp. 261—270). They would probably tend to collect about the surface of the members of the green vegetation, owing to the evolution of oxygen going on there; so that the red deposit would primarily appear round about the water-weeds and Algæ present. I do not, however, think that these deposits can be solely due to their agency, for in many cases there were very few bacteria evident; they seem to occur more commonly in later stages.

Although such ferruginous pools are very characteristic of Ceylon, they are not essentially tropical, and are found quite frequently in temperate regions. I have observed a similar reddish-brown deposit accumulating since several years in one of the small ditches on Sheen Common, Richmond (Surrey), and very much the same flora occurs associated with it as in the Ceylon pools. *Spirogyra, Œdogonium, Zygnema, Ulothrix, Conferva, Pleurotænium, Closterium, Netrium, Pinnularia, Navicula, Surirella,* and *Synedra lunaris* are the more important forms I have observed in the course of casual visits during the last three years. The species are apparently in many cases not identical with those found in the tropical pools, but that alters little in the astonishing similarity. During the period of observation, the granular deposit has steadily increased in quantity, while the Algæ have slightly decreased in numbers. Latterly, blue-green forms are putting in an appearance, which tallies with what I observed in Ceylon. The vegetation of these ferruginous pools thus constitutes one of the most striking cases of parallel between temperate and tropical algal formations that I have noticed, and probably stands isolated in this respect.

There is one more phenomenon noticed mainly in the pieces of water under discussion (also in the padi-fields), that may be briefly described. As above mentioned, pools without a noticeable Conjugate element are generally inhabited by Cyanophyceous growth,—often *Oscillaria.* A visit to such a pool in the early morning shows the muddy bottom covered with a more or less continuous film of the Alga, as is characteristic of the species of this genus. The film consists of a thin layer of algal filaments with a certain amount of mud between them. Soon after sunrise gas-bubbles make their appearance studded all over the surface of the films, whilst at many points the latter show signs of inflation indicating a similar development of gas-bubbles on the lower side. Soon after midday floating patches of *Oscillaria,* still with numerous adhering gas-bubbles, are found on the surface of the water, and a little patience enables one to follow the detachment and rising of such a patch to the surface. This state of affairs continues for the rest of the day, but early on the next morning there are again no floating patches of *Oscillaria,* which merely forms a film on the bottom. In the course of the day the same process repeats itself. This phenomenon is observed in waters which are shallow (and almost unshaded) and in which the midday temperature is very considerable (viz., 35° C.). The rise of patches of the films to the surface is, of course, due to the expansion of the gas-bubbles formed during the assimilation and respiration of the Alga, whilst the cooling of the water after nightfall involves a contraction and perhaps partial solution of these bubbles (and probably their consequent detachment), and this results in the sinking of the floating patches. The phenomenon is certainly no rare one in the case of Algæ capable of forming compact discs like *Oscillaria* (*cf.* also p. 227). Analogous, though not absolutely similar, is the rising of tangles of filaments, occasionally also to be seen in *Oscillaria*, though more frequent in green filamentous genera such as Conjugates, *Œdogonium, Cladophora,* etc.* The facts mentioned in this paragraph are also, of course, commonly to be noticed in temperate regions, but I am not aware that a daily rise and fall of such constancy ever occurs in our parts.

It seems probable that the Algæ in this case are merely the passive objects of a useless physical phenomenon, and that the daily rise and fall is of no service to them. But it seems well to point out that the rising of the oscillariate patch to the surface may be advantageous from the point of view of respiration and of assimilation (the latter owing to the considerable percentage of mud included in these oscillariate films). In any case the Alga only avails itself of a physical process, which would take place whether it was of service to it or not.

(iii) *Algæ of Marshes and Padi-fields* (*i.e.*, of ground permanently or periodically saturated with water which does not form definite pools).—There is very little difference on the whole between the algal flora of the padi-fields and that of the lowland pools and ditches, but the biological conditions are a little different, and it is a desire to emphasise these that leads me to hold the two distinct. The rice-fields are fully exposed to the strong sunlight, and the only (very scanty) source of protection is the frequent muddiness of the water. Desiccation is a regular periodic phenomenon, and periodicity would probably be found to be better marked in this flora than in the algal vegetation of any other Ceylon fresh-water. In their exposed habitat the rice-fields resemble the tanks, whilst the desiccation risks are analogous to those in the small pools and ditches. In correspondence with this the blue-green element is rather more pronounced than in the latter, although the Conjugates still very often have matters their own way. The latter are sometimes almost the only forms present in a padi-field, but just as often there is practically nothing but Cyanophyceous growth. *Œdogonium* is not so commonly met with as in the pools and ditches, but *Bulbochœte* is again quite a common form. The Desmid-flora is often very rich, whilst in one or two cases Diatoms (*e.g.*, *Gomphonema* attached to water-weeds) were present in exceptional numbers. A very characteristic member of the vegetation of the padi-fields is *Pleurosigma curvulum,* Pritch., though rarely present in any great quantity; another form frequently found in small numbers is *Fragilaria.* I did not meet with *Pithophora* or representatives of the Ulotrichales and Confervales in any of the padi-fields examined.*

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* *Cf.* Oltmanns, 'Morphologie und Biologie der Algen,' vol. 2, Allgemeiner Teil, Jena, 1905, p. 142; also H. Devaux, "Du Mécanisme des Échanges gazeux chez les plantes aquatiques submergées," 'Ann. Sci. Nat. Bot.,' 7th ser., vol. 9, 1889, p. 144. In the case of filamentous green Algæ, etc., the bubbles are retained between the tangle of filaments and, by their alternate expansion and contraction, cause the rising and falling of the tangles.

The periodicity of the algal growth in the rice-fields would be an interesting study, and would probably disclose interesting adaptations, which a brief examination such as mine could not possibly reveal. It seems that the first growth is almost always blue-green, and that the green element only appears subsequently when the rice and occasional water-weeds (especially Characeæ) afford a certain amount of protection against the strong light. Two fresh rice-fields at Matale showed practically nothing but blue-green forms, whilst a slightly more advanced field at Matara contained abundant *Oscillaria* intermingled with occasional filaments of *Spirogyra* and Diatoms and Desmids in some numbers. Old rice-fields often contained only very scanty Cyanophyceæ, but in their place quite a rich Conjugate and Diatom flora. It almost seems as though after the harvesting of the rice, and with approaching desiccation the blue-green element again gains the upper hand, since dry padi-fields often bear a filmy growth of some blue-green form on the stiff mud. I think the sequence just sketched out is near the actual one, but there are certainly occasional exceptions.

I met with very few examples of marshy ground other than that afforded by the padi-fields. Mr. Lewis kindly pointed out a fairly typical marsh to me about two miles inland from Kalutara; the ground was saturated with water and covered by a rich growth of mosses with *Urticularia, Nepenthes,* etc. The algal flora was that characteristic of such habitats in temperate regions, viz., a very rich Conjugate flora with numerous diverse Desmids and a certain number of Diatoms. Blue-green forms on the whole played a minor part. A small extent of low-lying swampy ground about half-a-mile to the east of Matara was covered by a rich growth of grasses, and was in most respects unlike the marsh at Kalutara. The algal flora though rich in Zygnemaceæ was exceedingly poor in Desmids, and Diatoms were common only as epiphytes. There was a considerable growth of a narrow species of *Œdogonium* at some points.†

(iv) *Algal Vegetation of the Rock-pools.*—At diverse points (Nalande, Dambulla, Kurunegala, Habarane) in the northern half of Ceylon one meets with huge black masses of basalt, which, besides forming the substratum of the local tanks, generally contain numerous small but deep pools, which often harbour an abundant growth of Algæ of a characteristic type, although the neighbouring tanks are generally very poor in littoral algal vegetation (often with a very rich Plankton, *e.g.*, Dambullawewa, Habaranewewa).* The temperature of the water in these pools is often high (31°·5 C. at Dambulla at 4 P.M.; 28°·5 C. at Hanwella at 8 A.M.; particularly high in the shallow pools at Kurunegala), but the daily range is certainly not very considerable, especially where the pools are deep, as at Dambulla and Nalande.† The pools are exposed to the full light, though the black rock-surface is probably a good absorber. The deeper pools, in particular, give one the impression of receiving relatively little light beyond the surface layers. The decided prevalence of Cyanophyceæ (especially in the shallower pools), however, speaks for the shading being less perfect than in the case of the pieces of water considered in Section (ii). The risk of desiccation is small in the deep pools, and it seemed as though many of the shallower ones owed their origin to springs. This would also mean good aeration, as is indicated by the whole aspect of the flora in many of the rock-pools; species characteristic of stagnant water are in most cases absent. The special conditions in these pools are thus: rocky substratum, high temperature of no considerable daily range (especially pronounced in the deeper pools), full exposure to light (except in case of deep pools), and probably fairly good aeration.

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* It may once more be pointed out that the specific constitution of the green algal flora is exactly the same as in the pools, ditches, and tanks (*cf.* p. 228 *et seq*).

† One or two further (brackish) marshes will be considered in conjunction with the estuarine flora in the second part of this paper.

The algal vegetation in the rock-pools either takes the form of a close pilose or fluffy covering on the rocky sides, or of a floating mass. More than half the number (18) of typical pools examined contained a blue-green flora with very few other forms; this is what we should expect under the dominant conditions. The chief representatives of the Cyanophyceæ are attached species of *Rivularia* and *Hypheothrix* (see below) and floating forms of *Anabæna* (sometimes also *Lyngbya*, mainly *L. majuscula*). Narrow species of *Oscillaria* are often present in minor numbers, but this genus plays a very unimportant part here in comparison to the pieces of water discussed in the previous sections. The absence of *Tolypothrix* is noteworthy. The blue-green element, though abundant, thus shows a very monotonous constitution, which is in striking contrast to the great diversity displayed by the green flora. This is very well developed in all the rock-pools not dominated by Cyanophyceæ, and also shows some uniformity in so far as it consists almost only of Conjugates and Protococcales. But, for the existence of forms belonging to these two groups, conditions must often be eminently suitable, for such pools have furnished a diversity of forms, which only finds its parallel in the upland pools. Diatoms (*Fragilaria, Navicula*) are rare—in fact often as good as absent (none in 11 of the pools), but Peridiniaceæ are often quite common amongst the members of the Conjugate vegetation. *Œdogonium*, if present at all, generally plays a very minor part amongst the Conjugates, although it attained considerable development in a pool at Kurunegala and one at Hanwella.* The species of this genus found in the rock-pools have generally rather broader filaments than the species inhabiting the waters previously discussed, although none of the forms could really be called broad. This feature may be connected with better aeration.

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* Similar masses of basalt are found round about other tanks (*e.g.*, Punchikekirawa, and between Kelawewa and Balaluwewa), and are no doubt covered by their waters in the wet season, so that the pools in them then become part of the tank. During the dry period, however, the pools are isolated and show a certain amount of individuality, though strongly dominated by the flora of the tank. They are considered in the present section, however, owing to certain similarities to the true rock-pools, as also are some pools observed in rock-quarries at Hanwella. The characteristic rock-pools (*i.e.*, those which are not in connection with a tank) are distinguished in the above description as the "typical" ones.

† It should be pointed out that the water of these pools is not only heated directly by the rays of the sun, but indirectly through the medium of the surrounding rock. The latter will retain the heat much more tenaciously, and this will serve to decrease the daily range of temperature in the pools in the rock, especially in the deeper ones.

The abundant Conjugate element comprises certain species of Zygnemaceæ and Desmidiaceæ, and although these forms have been shown to play a part in the minor waters of the lowlands generally, we shall find that they exhibit a markedly different aspect in the rock-pools. In the first place *Spirogyra* is mostly only a subsidiary form, in many cases, indeed, of isolated occurrence; only in three of the 18 typical pools examined did it play an at all important part, whilst in nine of them it was completely absent (even in some cases in which the other Conjugates flourished). Two of the three cases of abundant occurrence of *Spirogyra* are from the deep pools at Dambulla, and this lends support to the view above suggested of the efficacy of the dark rock-surface as a light-absorber and the consequent shaded character of the water in these deeper pools. The third case is from a pool at Kekirawa shaded by the neighbouring jungle. Of equal importance to *Spirogyra* are species of *Zygnema* which generally take its place; from no other waters in the island have I seen as much *Zygnema* as in the rock-pools. *Mougeotia* is completely absent, but in one case a considerable amount of *Gonatonema* occurred. In other cases filamentous Desmids (fig. 4, F and M) play a great part (species of *Desmidium, e.g., D. Aptogonium,* Bréb., *D. quadratum,* Nordst., *D. didymum,* Corda, and *Sphærozosma, e.g., S. secedens,* De Bary). *Desmidium* is particularly characteristic of these pieces of water, while other filamentous Desmids (*e.g., Hyalotheca* and *Gymnozyga*) which are common enough in the ordinary lowland pools, are almost or completely

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* The relative scarcity of species of *Œdogonium* in the rock-pools may have something to do with the absence of a suitable substratum. The young plants tend to be epiphytes on other filamentous Algæ or on water-weeds; the only filamentous forms present are Conjugates, and these are well known to apparently afford an unsuitable substratum for epiphytes, whilst water-weeds are rarely present. In the pool at Hanwella the *Œdogonium* (*O. calcareum ?*) was attached to the rocky base, but this is the only example of the kind noticed. At Kurunegala there was a little of a water-weed growing in the rock-pool, and this bore the *Œdogonium* in this case; in another rook-pool at the same place many of the decomposing plant-portions forming a sediment at its bottom bore a fair number of 2- or 3-celled young plants of the genus in question.

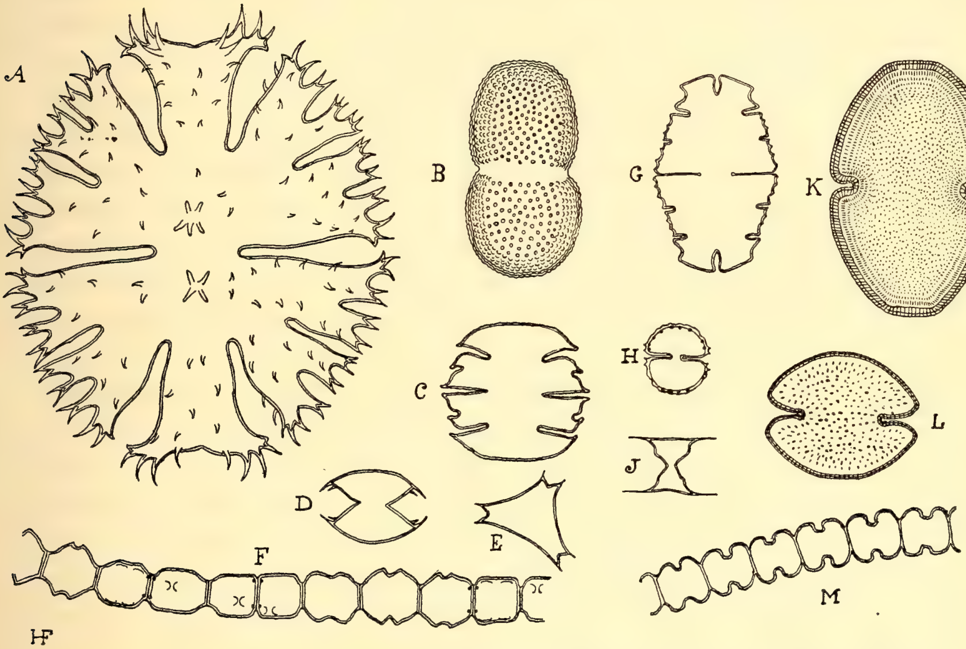

Fig. 4.—Illustration of the Types of Desmids characteristic of the Ceylon Rock-pools. The figures are intended to show the prevalent type, and in no way aim at giving an exhaustive representation of the great diversity of form presented by the Desmids of these pieces of water. A, *Micrasterias apiculata* (Ehrenb.), Menegh. var. (× 250). B, *Cosmarium decoratum,* West and West (× 250). C, *Micrasterias zeylanica,* n. sp. (× 250). D, *Staurastrum bifidum* (Ehrenb.), Bréb., side view (× 330 about). E, Ditto, end-view (× 330 about). F, *Desmidium quadratum,* Nordst. (× 330 about). G, *Euastrum zeylanicum,* n. sp. (× 560 about). H, *Cosmarium* (*cf. punctulatum,* Bréb.) (× 330 about). J, *Staurastrum aristiferum,* Ralfs ? (× 330 about). K, *Cosmarium pseudo-pachydermum,* Nordst. (× 250). L, *Cosmarium Lundellii,* Delp. forma (× 250). M, *Sphærozosma secedens,* De Bary (× 560 about).

wanting. The unicellular Desmids show similar characteristic features; thus species of *Closterium* are either entirely absent or at least rare, and when present they are mostly not those species which are found in the other lowland pools; *Euastrum* is also much rarer than in the latter, but *E. dideltoides*, West and West and *E. zeylanicum*, n. sp.* (fig. 4, G) were rather abundant in one case. On the other hand, species of *Micrasterias*, which are rare in the ordinary pools, play a great part in these pieces of water (*e.g.*, *M. apiculata*, Menegh., *M. zeylanica*, n. sp.,† fig. 4, C, etc.), whilst species of *Staurastrum* (*e.g.*, *S. bifidum*, Bréb., *S. aristiferum*, Ralfs ?) and *Cosmarium* (*C. decoratum*, West and West, *C. pseudopachydermum*, Nordst., *C. Lundellii*, Delp., *C. punctulatum*, Bréb. ?) are strikingly abundant in contrast to their scarcity in the clayey pools. *Pleurotænium* (*P. Trabecula*) is sometimes to be found in some quantity. Looking at the Desmid-flora of the rock-pools in its entirety, one is struck by the complexity of form of the diverse species in contrast to the relatively simple forms assumed by the Desmids of the ordinary lowland pools. This feature is illustrated both by the unicellular and filamentous species (*cf.* figs. 3 and 4), and is certainly very noteworthy (possibly due to aeration of the water ?) As already mentioned, numerous members of the Protococcales (*Scenedesmus*, *Pediastrum*, *Cœlastrum*, *Rhaphidium*, *Dimorphococcus*, etc.) sometimes accompany the Conjugate element in striking numbers, and in one or two cases Peridiniaceæ are quite common. The exceptional abundance of the flora in these rock-pools can only be referred to the high temperature of the water (perhaps also to the favourable conditions of aeration ?).*

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* *Diagnosis.—Euastrum zeylanicum*, n. sp.:—Cells small, about one and a-half times longer than broad, very deeply constricted. Apical incision first slightly widened and then narrowed to more or less of a point. Semi-cells pyramidate, with pointed basal angles; apex convexo-truncate, the margin on either side of the apical incision being straight or slightly curved. Lateral margins with two incisions, of which the one next the apex is deeper than the other; upper portion of lateral margins (*i.e.*, part between the apex and the first lateral incision) retuse; portions of lateral margins between first and second incisions and between second incision and median sinus denticulate. Median sinus very narrow and closed, slightly dilated at its inner end. Cell-wall smooth. Zygospore not seen.

Length 45—50 μ, breadth 30—35 μ, breadth of apex 16—18 μ, breadth of isthmus, 8 μ.

E. zeylanicum has some points of resemblance to species like *E. radiatum*, Turn., and *E. serratum*, Josh., but the differences are perfectly obvious.

† *Diagnosis.—Micrasterias zeylanica*, n. sp.:—Cells small, length generally slightly exceeding the breadth, rather deeply constricted; sinus open, triangular acuminate with a pointed inner termination; semi-cells three-lobed, lobes separated by incisions, which penetrate as deeply as the sinus, and are inclined towards the latter; ends of terminal lobe bluntly pointed; lateral lobes with two blunt processes, separated by a narrow and rather shallow, rounded depression, the process next to the sinus being horizontal, the other bent down towards the sinus; polar lobe widely spreading, slightly drawn out at each extremity into a horizontal or deflected process; apex flatly convex. Outline very faintly granular. Cell-wall smooth or minutely punctate. Zygospore not seen.

Length 60—65 μ, breadth 55—60 μ, breadth of polar lobe 50—55 μ, breadth of isthmus, 15—18 μ.

Micrasterias zeylanica belongs to that group of species which includes *M. oscitans*, Ralfs, and *M. incisa*, Ralfs, but is more complex than either of these. In this respect it comes nearer to *M. truncata* (Corda), Bréb., which is, however, much more abundantly incised.

Before concluding the description of the algal vegetation in the rock-pools, I wish to add a few remarks on the biology of the attached growth found on the rocky sides of these pools, and we may extend the discussion to the algal covering on submerged rock-surfaces in general. This attached growth is either an adhesive tangle or is composed of velvety tufts of pilæ. The former is often very compact, and consists either of a single species or more commonly of a number of closely interwoven forms. The basis in the latter case is generally blue-green, although green forms (*Œdogonium*, *Mougeotia*, *Microspora*, *Ulothrix*, *Stigeoclonium*) often make up a large part of the mass, whilst unicellular forms (Desmids, Diatoms, Protococcales) are frequently intermingled. Such adhesive tangles are, no doubt, composed of certain definite associations of species mutually dependent one upon another in certain respects, and extensive investigation would probably show that similar (in some cases perhaps identical) collections of species occur in different localities under the same influencing conditions. The advantage of such composite growth may lie in its forming a source of protection against the ravages of aquatic animals, but there may even be a more intimate relation involving the metabolic activities of the organisms concerned. For such associations of species I propose the term "*consortium*,"† one example of which has already been noticed in the *Lyngbya*-films found in Lakes Kantelai and Mineri (p. 227); *Pithophora*, with its associated epiphytes, may be mentioned as a second.‡ Consortia are, of course, to be found in every piece of water, and I hope at some later date to deal with those occurring in our waters.

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* It may be suggested that the great specific diversity and relative individual complexity presented by the algal flora in these pools (and especially in the deeper ones) may be, to some extent, due to an absence of the external conditions influencing algal growth in other tropical pieces of water. Especially in deep rock-pools desiccation probably never takes place; the temperature is not subject to much daily variation; the aeration is probably fairly adequate; and the light conditions are not extreme. These characters of the habitat are probably favourable to the growth of a large number of different forms, whose vegetation goes on undisturbedly. A considerable amount of variation is thus possible in these waters, and I should not be surprised if a relatively large number of new forms will be found in them as a result of a more extensive investigation.

† *Consortium* (Latin) = fellowship, participation.

‡ *Cf.* Fritsch, "Problems in Aquatic Biology, etc.," 'New Phytol.,' vol. 5, No. 7, 1906,

The pilose growth (fig. 5), so often found coating rocky surfaces in the tropical fresh-waters, is almost entirely blue-green, and presents features analogous to those noticed above in connection with the subaërial flora. The pilæ consist of a single or of several different species, and in the latter case we are again dealing with a consortium in which the interrelation could be determined to some extent. In one of the rock-pools at Dambulla the whole submerged surface of the rock was thickly covered with such pilæ. When some of these were carefully scraped off, a dark dot was seen with the naked eye at the base of each of them. Microscopic examination showed that this dot was due to little radiating colonies of *Rivularia* (fig. 5, B and D), whilst the remainder of the pila was constituted by a species of *Hypheothrix,* the bundles of filaments of the latter arising from in between the threads of the basal *Rivularia.* The branched bundles of the *Hypheothrix* are held together to form the tuft by a very narrow species of *Oscillaria* ramifying in all directions between the bundles of *Hypheothrix*-filaments. In the outer portion of each pila there was a considerable amount of mud and decomposing algal substance, which afforded shelter to a number of green unicellular forms (*e.g., Scenedesmus, Tetraedron*). The consortium here thus consists of a *Rivularia*, a *Hypheothrix*, and an *Oscillaria* with a few unicellular forms. It seems as though the *Rivularia* prepares the ground for the *Hypheothrix* which follows. A very similar pilose covering was found on the rocks in a small pond near the tank at Habarane, but the pilæ in this case were considerably longer. The basal *Rivularia* showed up very distinctly and, with a lens, its filaments could be seen radiating out in all directions (fig. 5, A). The species of *Hypheothrix* differed from the one observed at Dambulla, the filaments forming thick unbranched bundles, each constituting a single pila; as a result, there was no binding *Oscillaria* in this case. The slight differences, however, do not detract from the interest of the very considerable analogy. *Rivularia* plays a very important part in all the rock-pools, and in some of them was found growing on every conceivable object (including loose sediment).

As above mentioned (p. 228) the rocks round the edge of Lake Kantelai were covered by a thick pilose blue-green growth. This, again, consists of a species of *Hypheothrix,* the bundles of which are bound together by a delicate *Oscillaria,* but there is no basal *Rivularia* in this case. At the base of many of the pilæ a dark dot is indeed to be seen (fig. 5, C), but microscopic examina-

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p. 157. Every piece of water contains forms, which are in part at least dependent one on the other, be it from the point of view of substratum, of shading, of transference to a suitable position in the water, or of protection from animal attacks: and all such associations of species occur together as a result of necessity.

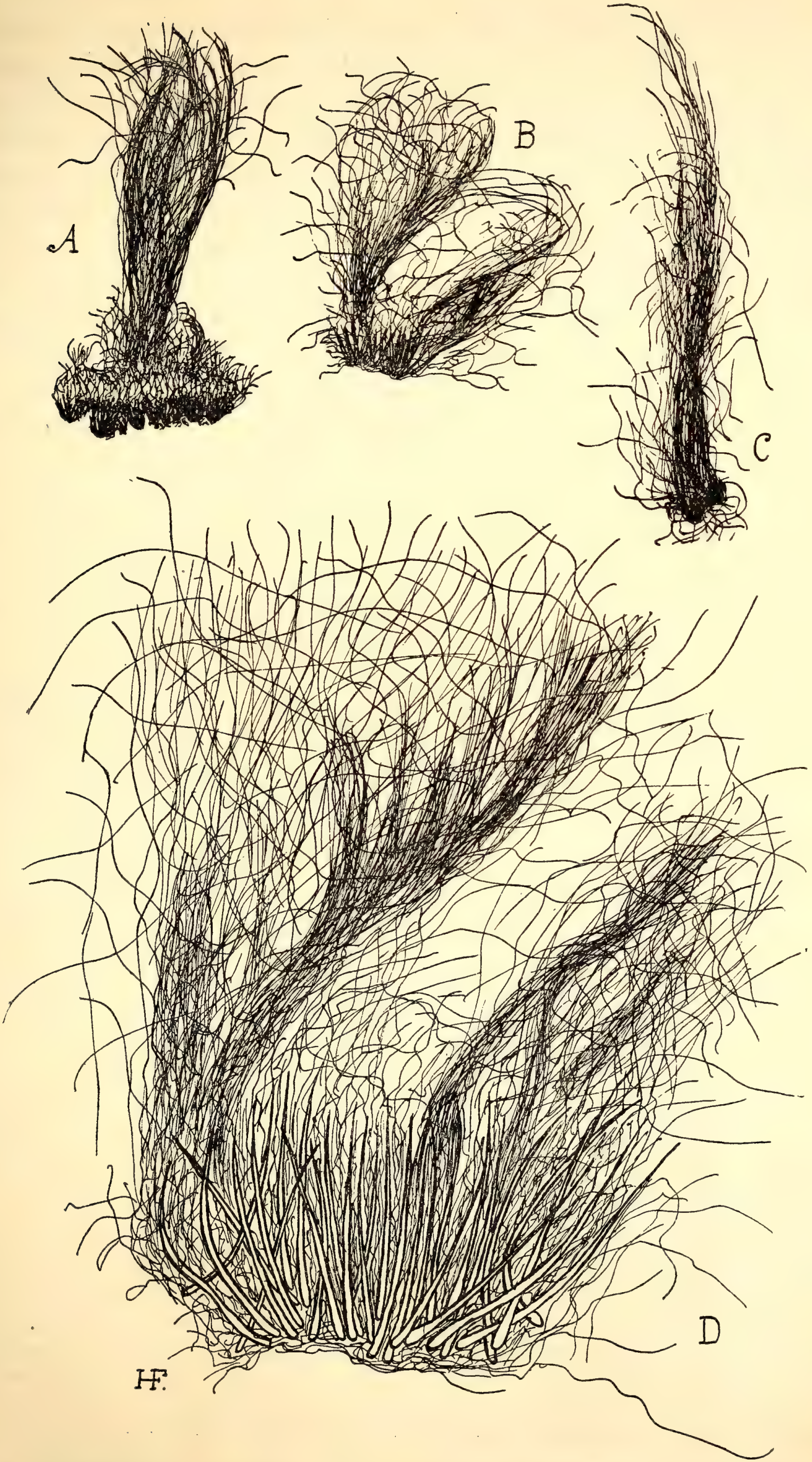

Fig. 5.—Illustration of Colonisation of Submerged Rock-surfaces. A ($\times 8\frac{3}{4}$), Single tuft from pilose growth on submerged rocks of a small pond near the tank at Habarane; growth of *Rivularia* at base of *Hypheothrix*. B ($\times 13\frac{1}{3}$), Single tuft from pilose growth on sides of rock-pools, Dambulla. D (×35), the same enlarged. C (×nearly 8), Tuft from pilose growth on submerged rocks in Lake Kantelai; the dark mass at the base is due to air. Full descriptions of all the figures in the text.

tion shows this to be due to a collection of air at these points. Pressure on the cover-glass breaks up this air into numerous small bubbles, which speedily become dispelled into the surrounding water, and after this process the black dot at the base of the pila has vanished. Perhaps we have here an air-bubble which was originally clinging to the rock-surface and formed the base for a growth of *Hypheothrix*, since some algal forms are occasionally to be found attached to the surface-film of air-bubbles in the water.*

In concluding the discussion of this subject, I should like to point out that blue-green Algæ in particular are noted for the frequent occurrence of dense collections of very diverse forms, which have been one of the most fruitful sources of the establishment of cases of so-called polymorphism. Had the observers who dealt with this subject attacked the physiological problems involved in these close associations of individuals, far more good would have been done than in the proclamation of cases of polymorphism on superficial data. For such collections of blue-green Algæ are certainly nothing more than highly interesting consortia.

(v) *Algæ of Wells and Springs* (*i.e.*, aerated but standing water).—I was only able to examine three examples of the algal growth in wells, but they have proved very instructive. The material is derived from three different localities (Nalande, Matale, and Ambalangodda), so it may be looked upon as fairly typical for the island. The fundamental point about these pieces of water is their good aeration. The water is generally at some considerable depth below the top of the well and is consequently not exposed to a very strong light, nor is its temperature probably in any way as high as in the more exposed waters we have previously discussed. The substratum is stone or wood work. The prevailing conditions are thus very marked and uniform and produce a very typical algal flora.

The predominant forms are species of *Pithophora*, which were present in all three cases and in two of them absolutely dominated the algal flora. In two of the three wells at least there was also a certain amount of other Cladophoraceæ (*Cladophora*, *Rhizoclonium*). Since the material was in all cases sterile and often covered with epiphytes, the discrimination of *Cladophora* and *Pithophora* was not easy; the large mass of the Cladophoraceæ present, however, showed the vegetative characters described by Wittrock† as characteristic of *Pithophora*. In two of the wells there was a certain amount of *Spirogyra* presenting the usual features, whilst blue-green forms (*Lyngbya*, *Oscillaria*) are always present, sometimes in quantity. *Œdogonium* was only observed in the well at Matale, where it occurred as an epiphyte on the *Pithophora.*

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* *Cf.* also Fritsch, "The Structure and Development of the Young Plants in *Œdogonium*," 'Annals of Botany,' vol. 16, No. XLIII, 1902, p. 473.

† *Cf.* Wittrock, in 'Nov. Act. Reg. Soc. Sc. Upsala,' Ser. 3, 1877, p. 4.

The only other forms of any importance are Diatoms, which were present in enormous numbers in two of the wells; in the third (at Ambalangodda) there were practically none, which is due, perhaps, to the main mass of algal growth found here being above water-level and consequently exposed to a higher temperature than in the other two cases. In the wells at Nalande and Matale the Diatoms occurred mainly as epiphytes on the *Pithophora;* in the former case there were epiphytic *Gomphonemas* and *Synedras* and loose floating tangles of *Fragilaria* (with intermingled blue-green forms and *Synedra*), whilst in the latter, except for a few *Synedras,* all the Diatoms were epiphytic (*Achnanthes, Gomphonema,* and *Synedra*). Nowhere else in the lowland fresh-waters was such a striking abundance of Diatoms observed, and I think this feature is here rather a result of the colder water than of the good conditions of aeration. *Fragilaria* is, however, a genus which prospers quite well in warm water (*cf.* below), and the conditions influencing its presence or absence are very unclear.

The vegetation of these wells is thus characterised by a Cladophoraceous and Diatomaceous element and shows a very uniform composition. The conditions are, of course, exceptionally favourable for Cladophoraceæ, for not only is the aeration adequate, but the lower temperature of the water involves a greater power of dissolving the necessary gases. The wells are just the kind of habitat in which we should expect to find Cladophoraceæ flourishing in the tropics (*cf.* p. 230), if anywhere. And their predominance in such waters furnishes, to my thinking, a striking confirmation of the causes suggested above for the scarcity of this order in other tropical fresh-waters.

A few words may be added on the vegetation of the hot springs at Kannia, near Trincomalie, although they will be dealt with more fully elsewhere. In hot springs we have aerated water at a high temperature, and this is the marked distinction between them and the wells. The springs at Kannia are roughly of two temperatures, viz., 37° C., and 40° to 41° C., and these two different temperatures appear to call forth an entirely distinct algal vegetation in the respective springs. Those at the higher temperature have the stone sides above and below water-level covered with a continuous tangled or filmy growth of blue-green Algæ (mainly *Lyngbya* and *Scytonema*), whilst the less hot springs (37° C.) have a brown, fluffy covering of *Fragilaria* on their sides. This difference is very well marked, especially as regards the *Fragilaria,* which scarcely appears at all in the hotter springs. Since there is no other obvious factor than the difference of temperature to explain this diversity, we must look upon the higher temperature (only 4° C. above the other, however) as being the cause of the absence of *Fragilaria* in the hotter springs.

(vi) *Algal Vegetation in the Small Pools of the Uplands.*—The vegetation of the numerous small pools occurring round about Nuwara Eliya (especially on the so-called Moon Plains and along the route from Nuwara Eliya to Hakgalla) formed a ready means of study and furnished interesting materials for a comparison with the lowland pools. The differences are just what we should expect to find in view of the different conditions. As Mr. Nock kindly informed me, these small upland pools dry up for about a month during the dry period. Their water is generally almost quite clear, but stagnant, and the midday temperature (October 29) in four cases was found to be 19°·5 C. The bottom and sides in some cases seemed to consist of a white sandy loam, but in other cases they were clayey. Some of the pools are of considerable depth, others, again, shallow. Except for the slight shade afforded by the growth of neighbouring grasses, etc., the only protection from the strong light is found in the frequent occurrence of water-weeds, but some of the pools are quite destitute of such growth. The important conditions are the fairly low temperature, the clear water, and the probably rather intense illumination.

In very many of the pools the algal vegetation takes the form of a thin, almost transparent filmy growth covering the abundant water-weeds, and occasionally floating on the surface of the water. These films are so delicate that they break up as soon as one attempts to remove the weeds bearing them from the water, and the only way to collect them is to push the plants into the collecting-tube under water. This character of the films is due to their being composed almost entirely of unicellular forms, of which Desmids and a limited number of Protococcales are the most important. Algal growth of this kind is common enough in temperate regions,* and it is interesting to find it developed here, in this pseudo-temperate part of Ceylon; as far as I have seen, such films are not present in the lowland waters. A considerable part of the films is composed of mud and decaying organic particles, and this matter and the algal cells are no doubt held together to form a coherent whole by the considerable masses of mucilage† excreted by the Algæ forming the films. This mucilage is, however, very delicate, in most cases not being obvious without the help of stains, and this accounts for the very delicate and frangible nature of the films. Although films of this type have frequently been described, their origin has, as far as I am aware, not yet been adequately explained. It may be noticed in this connection that the Desmids most involved are capable of slow movement with the help of the excreted mucilage,* and they probably tend to collect in considerable numbers on the surface of the water-weeds, attracted, perhaps, by the oxygen liberated during their assimilation. The prolific division of the Desmids thus established will soon lead to an accumulation of numerous individuals, whilst their mucilage-investments will flow together and form a whole. The frequent inclusion of Protococcales and of muddy particles in these films is not difficult to understand on this assumption, nor the occasional presence of Diatoms. Whether these composite films can be regarded as a true consortium must be left to further investigation.

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* *Cf.* Messrs. West and West, "A Monograph of the British Desmidiaceæ," vol. 1, Ray Society, 1904, p. 16.

† See especially B. Schröder, "Untersuchungen über Gallertbildungen der Algen," 'Verh. Nat.-Med. Ver.,' Heidelberg N. F., vol. 7, 1902, pp. 139—196, Plates VI and VII.

Turning our attention now to the specific constitution of these films, we have, in the first place, to notice that the blue-green element is practically unrepresented; isolated filaments of *Oscillaria* are sometimes present, and in one case the films included a number of colonies of *Chroococcus.* As a rule, however, Cyanophyceæ are sought for in vain, and from this point of view alone these upland pools acquire an aspect rather distinct from those of the lowlands. The Desmids are the most important forms (species of *Cosmarium*, *Euastrum*, *Closterium*, *Micrasterias*, *Penium*, *Xanthidium*, *Pleurotænium*, etc.), but filamentous species of this group are exceptionally rare (only *Spondylosium*). This latter point is not difficult to understand in the case of the films, for here the absence of filamentous Desmids is probably due to the immobile character of these forms which, on the above assumption of the origin of the films, would be excluded from participation in their formation. A more puzzling feature, however, is the great scarcity of filamentous Desmids generally in the upland waters examined (*cf.* also p. 238, footnote). This is a second striking point of difference between the upland and lowland pools, for in the latter, where the conditions are favourable, filamentous Desmids are quite a prominent feature. It may be that the diversity is due to the different amounts of dissolved oxygen in the water in the two cases. We have only the analogy of the colonial Protococcales to go by, but in some of these (*Scenedesmus*, *Cœlastrum*) Senn† found that colonies were formed when there was a scarcity of oxygen, whereas an abundance of the latter led to the formation of isolated cells. Possibly, then, the conditions prevalent in the lowland pools are such as to favour the existence of filamentous Desmids, while in the uplands the reverse is the case. The lowland pools certainly afford striking examples of the filamentous tendency amongst the Desmids

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* B. Schröder, *loc.* cit., pp. 157—159, Plate VII, figs. 3, 4, and 10*a*.

† "Ueber einige koloniebildende einzellige Algen," 'Bot. Zeitung,' vol. 57, 1899, p. 97.

(*e.g.*, the occurrence of *Micrasterias foliacea*, one of the few forms of this genus that has assumed the filamentous condition). It is, of course, quite possible that there are other more obscure causes for the great scarcity of filamentous Desmids in the upland pools. With reference to the above suggestion, it may be pointed out that filamentous Desmids are, on the whole, also a rather rare feature in our waters (except *Hyalotheca dissiliens* (Sm.), Bréb., and *Gymnozyga moniliformis*, Ehrenb.), which may be due to the higher percentage of dissolved oxygen.

Of the unicellular Desmids, *Cosmarium*, *Euastrum*, and *Closterium* play the most important part in the composition of the films, one of these three being generally the dominant form. In some cases one or other of these three genera is almost alone represented, and we obtain a practically pure culture of a number of species of the respective genus. In other cases, however, the films show a more varied aspect, containing most of the Desmid genera above mentioned, though many of them only occur in subordinate amount. Some of the films are composed of Desmids only; in others various Protococcales (*Raphidium, Crucigenia, Cœlastrum, Eremosphæra, Scenedesmus,* etc.) play a more or less important part. No doubt the presence or absence of these latter forms depends on certain conditions—possibly on the quantity of organic substance dissolved in the water.* Diatoms occasionally occur in some quantity, but are often almost completely wanting.

The algal vegetation in the small pools of the uplands does not, however, always take the form of these films. In some cases one finds a granular or flocculose covering on the bottom, whose composition is quite identical to that of the films. A mass of filamentous green Algæ floating on the surface (*Spirogyra, Mougeotia, Ulothrix,* frequently associated with Desmids) is not very common. In a few cases various blue-green Algæ are found, mostly as a fluffy coating to weeds, etc. I was unable to determine whether they are only developed in particularly exposed localities. Lastly, I may mention that I met with a species of *Vaucheria* in the water of a small pool in the Hakgalla Botanic Gardens. This is the first record of a fresh-water species of *Vaucheria* from Ceylon.

Meagre as the facts thus are that I was able to derive from my brief study of the vegetation of these upland pools, they yet point to a marked diversity as compared with the lowlands and a distinct similarity to the algal flora of temperate regions. These facts point to the temperature of the water (and indirectly the lower percentage of dissolved oxygen) as one of the chief factors influencing tropical algal vegetation.

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* *Cf.* Schmidle, "Aus der Chlorophyceen-Flora der Torfstiche zu Virnheim," 'Flora,' vol. 78, 1894, p. 64.

Experiments made to determine the Conditions under which "Specific" Bacteria derived from Sewage may be present in the Air of Ventilating Pipes, Drains, Inspection Chambers and Sewers.

By MAJOR W. H. HORROCKS, R.A.M.C.

(Communicated by Colonel David Bruce, C.B., F.R.S., R.A.M.C. Received January 11,—Read February 7, 1907.)

Most sanitarians at the present time believe that when sewage is in a putrefactive condition and gas bubbles rising through it are bursting at the surface, bacteria may be carried into the air of drains and sewers. It is also considered possible that when sewage has dried on the surfaces of pipes, bacteria may be separated as dried particles and carried some distance by currents of air passing through the pipes.

The following experiments were designed to ascertain whether there are any scientific facts on which to base these beliefs, and may be arranged in three groups.

GROUP 1.—*Experiments to determine whether Specific Bacteria are ejected into the Air by the bursting of Bubbles at the surface of Sewage.*

In the first series of experiments, sewage obtained from a main sewer in Gibraltar was inoculated with a rich emulsion of *B. prodigiosus* and then poured into a deep glass jar so as to form a layer at the bottom about 2 inches deep. At a height of 4 inches from the surface of the sewage two Petri dishes containing nutrose-agar were fastened, the medium facing upwards, to wire tripods, which were then firmly wedged in the bottom of the dish. A glass cover was then put on the dish and the fluid gently shaken by a horizontal movement, until a layer of bubbles formed on the surface of the sewage. This procedure was followed at intervals for three days, the plates were then taken out and incubated at 22° C. After seven days' incubation no signs of the *B. prodigiosus* appeared. Only one colony composed of cocci derived from the air was seen. This experiment was repeated again and again, but invariably with negative results.

The sewage was next inoculated with a rich emulsion of *B. typhosus,* and litmus-lactose-nutrose-agar plates were fastened to the tripods. The dish was shaken as before, the plates were then removed and incubated at 37° C. No signs of *B. typhosus* or *B. coli* were observed after incubation for one week.

Old sewage, smelling strongly of sulphuretted hydrogen gas, was then placed in the dish and the experiments were repeated as before. It was thought that gas bubbles would form more readily in old than in fresh sewage; this proved to be the case, but the special organisms added to the sewage never appeared in the plates.

Soapy water from a lavatory basin was then inoculated with *B. prodigiosus* and freely shaken in a glass bottle until it was permeated with bubbles; the fluid was then transferred to the glass dish, the plates fastened to tripods were rapidly placed *in situ*, and the cover put on. Twenty-four hours elapsed before all the bubbles dispersed, the plates were then taken out and incubated as before, but no signs of the *B. prodigiosus* appeared.

In the above experiments there were no air currents circulating above the sewage, and the bacteria could only be ejected by the bursting of bubbles. The results appear to show that independently of air currents, bacteria will not be ejected to a height of 4 inches by the bursting of infected bubbles.

The next series of experiments were made with the apparatus shown in fig. 1. Plates of nutrose-agar were suspended by means of wire cages in the vertical pipe, the uppermost plate being 9 feet above the water in the trap. Soapy water, inoculated with an emulsion of *B. prodigiosus*, was shaken up in a glass stoppered bottle until the whole fluid was permeated with bubbles, the contents of the bottle were then poured into the trap until it was filled. Under these conditions currents of air passing up the vertical pipe were able to carry bacteria separated from the fluid by the bursting of bubbles. The plates were removed at the end of two hours and incubated at 22° C. The result was that colonies of *B. prodigiosus* appeared in every plate at the end of 72 hours.

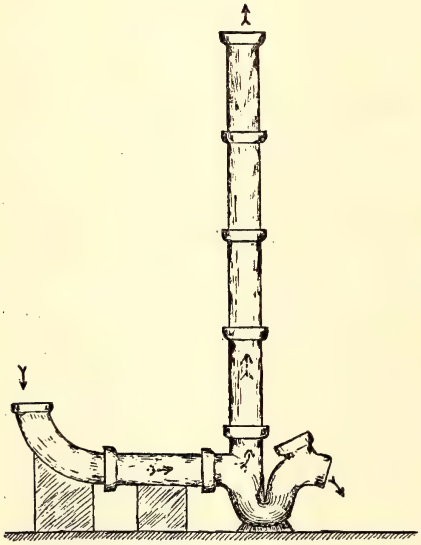


Fig. 1.

The last experiment of this series was made with a catch-pit on the storm-water system of the town. Complaints having been made of foul odours arising from the pit, the hinged cover was thrown back, and it was then seen that bubbles were rising through the water retained in the pit as a result of fermentation processes going on in the mud at the bottom. The pit contained

180 gallons of surface water at the time. An emulsion of *B. prodigiosus* was poured into the water and plates of nutrose-agar were then suspended as shown in fig. 2. Twenty-four hours later the plates were removed and incubated at 22° C. for two days, when numerous colonies of *B. prodigiosus* were found in all the plates. Control plates of nutrose-agar, exposed to the air outside the pit, did not show any signs of the special organism used in the experiment.

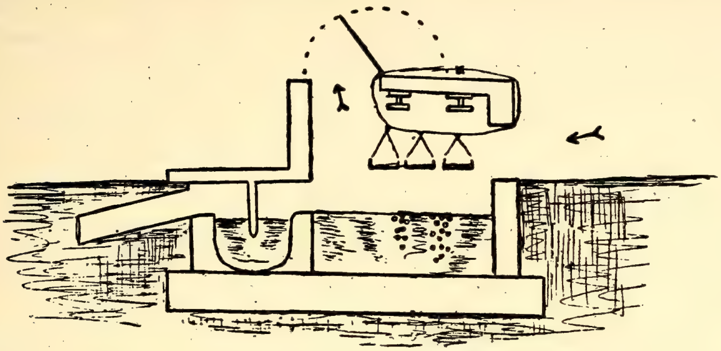


Fig. 2.

These results show that bubbles rising through stagnant water may eject bacteria, which will be carried away by currents of air passing over the surface of the fluid.

Group 2.—*Experiments to determine whether Bacteria dried on the surfaces of Pipes are likely to be separated and carried by Currents of Air passing through the System.*

The apparatus shown in fig. 3 was employed. Three 2-ft. lengths of 6-inch piping were laid on the ground and a rich emulsion of *B. prodigiosus*

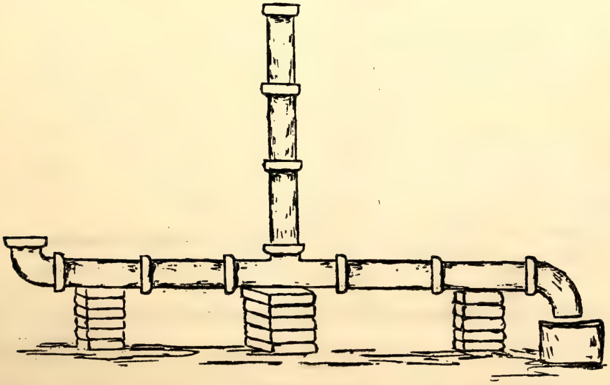


Fig. 3.

was poured inside each of them. The pipes were then rolled backwards and forwards until the fluid was uniformly diffused over the inner surface of each pipe. Twenty-four hours later the pipes, being perfectly dry, were fitted together with clay joints so as to form the vertical pipe of fig. 3. Nutrose-agar plates were then suspended in the pipe and sewage was allowed to flow through the horizontal piping at a rate not exceeding 3 feet per second for 20 minutes. The plates were then removed and incubated at 22° C.

At the end of 72 hours, all the plates were found studded with colonies of *B. prodigiosus.* The experiment was repeated several times, and on each occasion the same result was obtained. As the sewage passing through the horizontal pipes did not contain *B. prodigiosus,* and control plates exposed to the air were also free from this organism, it is fair to assume that the currents of air produced by the passage of sewage through the horizontal pipes carried up dried particles of *B. prodigiosus* detached from the walls of the vertical pipe.

GROUP 3.—*Experiments to determine whether Specific Bacteria are ejected into the Air of Drains, Sewers, etc., from Sewage flowing under Normal Conditions.*

In the first series of experiments, the trap of a 6-inch disconnecting trap was filled with sewage, and two lengths of 6-inch drain piping, having a junction bend turned upwards, fixed at one end, were fitted horizontally into the house side of the trap. Similar lengths of drain piping, but with the junction bend turned downwards, were also fitted to the sewer side of the trap. The vertical portion above the trap was lengthened by the addition of three 2-ft. lengths of 6-inch drain piping. The apparatus is shown in fig. 4.

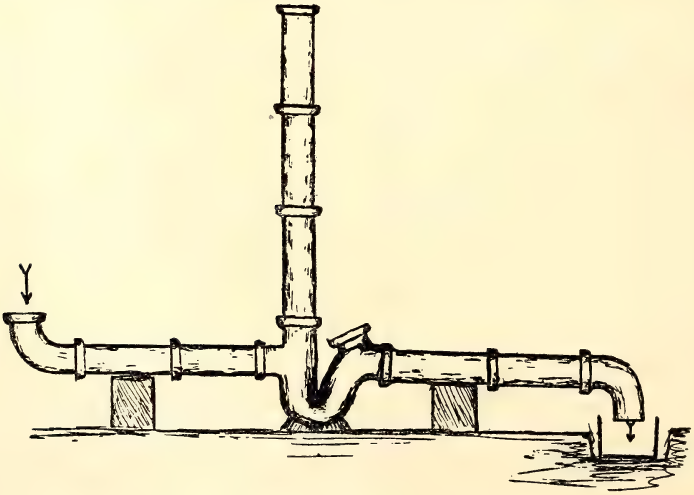


FIG. 4.

Three litmus-lactose-nutrose-agar plates were next suspended with the media facing upwards in the vertical pipe by means of wire cages, the uppermost plate being about 6 feet 9 inches above the sewage in the trap. Three gallons of sewage, taken from a main sewer in Gibraltar, were then inoculated with a rich emulsion of the *B. typhosus*, and poured down the bend on the house side of the trap. The sewage passed through the apparatus at a rate not exceeding 3 feet per second, and was received in a bucket placed under the bend on the sewer side of the trap. The inoculated sewage was passed through the trap in the same manner on two successive days for about half an hour. The plates were then removed and incubated at 37° C. for 24 hours, when numerous transparent blue colonies resembling those of the *B. typhosus* were seen in each plate. Several of the colonies were tested with anti-typhoid serum (horse) diluted 1—100 and, agglutination occurring at once, the colonies were planted out on agar slopes. The growths thus obtained were examined as to morphology and Gram staining, and then planted out in the usual media. The following results were obtained :—

Medium.	Result of incubation at 37° C.
Glucose-peptone	Acid, no gas.
Lactose-peptone	Unchanged.
Maltose-peptone	Acid, no gas.
Cane-sugar peptone	Unchanged.
Starch-peptone	Unchanged.
Neutral red	Unchanged.
Litmus-milk	Very faint acidity, no clotting.
Peptone-water	No nitroso-indol reaction.
Potato	Colourless growth.
Proskauer and Capaldi, No. 1	No growth.
Gelatine	Thin transparent growth, medium not liquefied.
Nitrate-broth	Reduced to NO_2.
Morphology, etc.	Small motile bacillus.
Gram staining	Decolorised.

The bacilli were agglutinated by an anti-typhoid horse serum diluted 1—500.

A portion of the agar growth derived from one colony was then emulsified in water and injected subcutaneously into a guinea-pig. As a control, a similar emulsion of the *B. typhosus* used to inoculate sewage was injected into a second guinea-pig of approximately the same weight. At the end of three weeks the sera of both guinea-pigs agglutinated, in a dilution of 1—100, the stock culture of *B. typhosus*.

It is plain that in this experiment the *B. typhosus* was ejected from the sewage to a height of 6 feet 9 inches.

Further experiments on the same lines were then carried out with the *B. prodigiosus*, an agar growth emulsified in water being added to the sewage. The same apparatus was used as in the previous experiments, the vertical pipe being gradually lengthened by the addition of 2-ft. lengths of 6-inch piping. The *B. prodigiosus* was recovered from the plates suspended 8 feet 9 inches and 11 feet 9 inches above the sewage in the trap.

It was thought that possibly the resistance produced by the passage of the sewage through the trap might have caused the projection of the special bacteria employed into the air contained in the vertical pipe.

Accordingly, in the next series of experiments, the trap was removed and the apparatus fitted up as shown in fig. 3. Plates of nutrose-agar were suspended in the vertical pipe, and the sewage mixed with *B. prodigiosus* was made to flow at a rate not exceeding 3 feet per second, through the horizontal pipes, which were never more than half filled with the sewage. The special organism was again recovered from plates suspended 11 feet 9 inches above the sewage in the trap.

As in all the above experiments, a very rich emulsion of the special organism, such as would never be found under natural conditions, was added to the sewage, it was determined to repeat the experiments, employing only 1 c.c. of the emulsion, representing one-ninth of the growth on an agar slope after 48 hours' incubation at 22° C., to inoculate the sewage. The same results were obtained as when the rich emulsion was used.

It is evident from these experiments that special bacteria can be ejected from flowing sewage independently of the resistance offered to the flow by the disconnecting trap. But as the plates were left in the vertical pipe for more than 24 hours, it is possible that the colonies in the plates might have been derived from particles dried on the surfaces of the pipes. In order to exclude this source of the bacteria, the experiments were repeated again, but the plates were withdrawn immediately the sewage had ceased to flow, each experiment only occupying 20 minutes. As before, colonies of *B. prodigiosus* were found in all the plates, showing that they must have been produced independently of dried particles carried by currents of air. As there were very few bubbles visible to the naked eye in the flowing sewage, it is not very probable that all the microbes found in the plates were ejected into the air by the bursting of bubbles. I think it is possible that many of the colonies were caused by the ejection of minute infected droplets from the flowing sewage. Gross splashing is out of the question, as the sewage was flowing at a comparatively slow rate, and plates were found infected at a height of 11 feet 9 inches above the surface of the fluid.

The next series of experiments were undertaken to test the value of the disconnecting trap as a means of protecting a house drainage system from specific bacteria present in the air of the sewer into which the house drain discharges.

The apparatus employed is shown in fig. 5. It was a combination of the arrangements used in the experiments already described. The straight run of piping, with the vertical pipe attached to it, represents the sewer and an attached ventilating pipe; joining the sewer is the house drain, the air in which is separated from that in the sewer by the usual disconnecting trap. The vertical pipe above the trap represents the air inlet or outlet, as the case may be, of the house drainage system. Plates of nutrose-agar were suspended in both vertical pipes. Sewage inoculated with *B. prodigiosus* was then allowed to flow at a rate not exceeding 3 feet per second through the pipes representing the sewer. The flow of sewage was continued for half an hour on two successive days; the plates were then withdrawn and incubated at 22° C. Colonies of *B. prodigiosus* were found in the plates placed in the ventilating pipe of the sewer, but none were present in the plates placed in the ventilating pipe above the disconnecting trap.

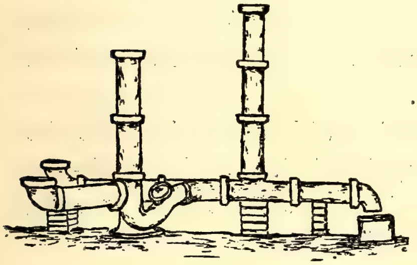


Fig. 5.

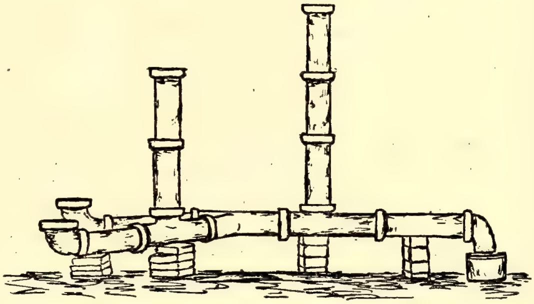


Fig. 6.

The experiment was repeated again, but during the flow of the infected sewage through the sewer the disconnecting trap of the house system was repeatedly flushed with 3 gallons of sewage. It was thought that during the flushing of the trap, microbes in the sewer air might possibly enter the house drainage system. This never occurred, the plates on the house side of the disconnecting trap never showed any colonies of *B. prodigiosus*.

The vertical trap was then removed and the apparatus arranged as shown in fig. 6.

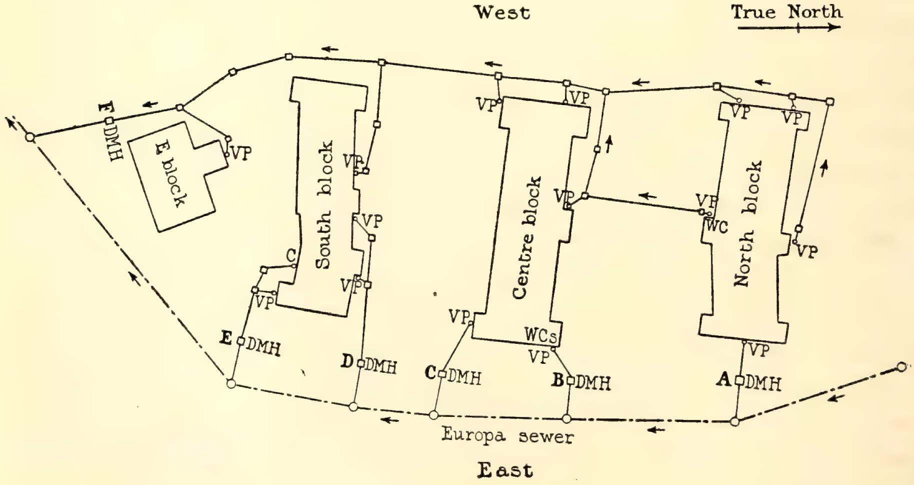
Inoculated sewage was made to flow through the sewer as before, and every five minutes a 3-gallon flush was passed through the house drain. Under these conditions colonies of *B. prodigiosus* appeared in the ventilating pipe of the house system as well as in that of the sewer. These results show that a disconnecting trap prevents microbes present in the sewer air from passing into the house drainage system.

The next series of experiments were made with actual drainage systems.

In the first experiment a newly laid system of a house in the town was tested by suspending plates in the junction and disconnecting chambers. A plate was also fastened in front of the air inlet of the system, the air inlet being provided with a mica valve. An emulsion of *B. prodigiosus* was placed in the pan of the W.C. of the house, which was then flushed out. The plates were removed 24 hours later and incubated, with the result that colonies of *B. prodigiosus* were found in every plate. "Air" plates exposed showed no signs of the special organism used in the experiment.

The second experiment was made at the military hospital, plates being suspended in the top of a 6-inch ventilating pipe connected with a W.C. in the basement. The plates were 50 feet above the ground level. A rich emulsion of *B. prodigiosus* was placed in the pan of the W.C., and the contents were then flushed in the usual manner. At the end of 24 hours the plates were removed and incubated for 48 hours, when all the plates were found studded with colonies of the special organism.

The third experiment was made in connection with the drainage at the east end of the Centre Block of the military hospital. At the north-east corner of the block there is a ventilating soil pipe, 6 inches in diameter, receiving the contents of a row of W.C.'s placed in an annexe on the first floor of the hospital. The soil pipe is connected by a 6-inch drain to a disconnecting chamber (B), which is 10 feet deep, and discharges its contents by a short drain into a 9-inch sewer, running along the Europa Road. At the



south-east corner of the block there is a similar disconnecting chamber (C), which receives the drainage from a W.C. in the basement and is also connected to the Europa sewer. There are six inspection chambers, with ventilating covers at the road level, and a 6-inch ventilating pipe on the Europa sewer, between the Centre Block and the Europa Pass Barracks, a distance of some 200 yards (see Plan of hospital drainage). Plates were suspended in the top of the hospital soil pipe, at the north-east corner, some 30 feet above the junctions of the W.C.'s, in the disconnecting chambers (A), (B), and (C), in all the inspection chambers connected with the Europa sewer and in the top of the ventilating pipe of the sewer. A rich emulsion of *B. prodigiosus* was then placed in each of the pans of the W.C.'s, which were then flushed. Twenty-four hours later the plates were removed and incubated at 22° C. After 48 hours, all the plates, except those in disconnecting chamber (A), were found studded with colonies of the special organism employed. Plates exposed on the ground close to the Centre Block for 24 hours were also incubated, but no signs of the *B. prodigiosus* appeared in them.

Judging by the results obtained with the experimental installations, it was expected that the special organism would be found in the hospital soil pipe, disconnecting chamber (B), and in the chambers and ventilating pipe of the sewer. But as the disconnecting chamber (C) at the south-east corner of the Centre Block is not in any way connected with the W.C.'s receiving the emulsion of the *B. prodigiosus*, and is separated from the Europa sewer by a modern disconnecting trap, the special organism should not have appeared in this chamber. The experiment was repeated again three times, and, in addition to the places already mentioned, plates were also suspended in the disconnecting chambers (D) and (E) connected to the east side of the South Block. The plates were left *in situ* for 24 hours, and then incubated as before. Colonies of *B. prodigiosus* appeared in the same places as in the previous experiment, but none were found in the chambers (D) and (E).

It was then evident that there must be some means by which the disconnecting chamber (C) was placed in communication with the Europa sewer. A careful examination of the bottom of the chamber was made, and it was found that there was no plug in the cleaning arm of the disconnecting trap; consequently, the chamber was in direct communication with the air in the sewer. Plugs were found firmly fixed in the cleaning arms of the disconnecting traps in the chambers (D) and (E), and prevented micro-organisms in the sewer air from entering the chambers.

The cleaning arm in the chamber (C) was then firmly plugged and the experiment repeated. The result was that colonies of *B. prodigiosus* appeared in the plates placed in the hospital soil pipe and in the chamber (B), but none appeared in the plates suspended in chamber (C), proving conclusively that the patent cleaning arm had been the channel through which the *B. prodigiosus* entered this chamber in the previous experiments.

As a control of the above experiments, plates were next placed in the inspection chambers connected with the west end of Centre and South Blocks. The contents of these chambers are removed by a separate drain, which passes along the west of the hospital into the disconnecting chamber (F), close to the south gate of the hospital. No colonies of *B. prodigiosus* were found in any of the chambers, although the plates were treated precisely as in the previous experiments. A fortnight later the experiment was repeated, but on this occasion the pan of a W.C. connected with the drain on the west side of the hospital was inoculated with *B. prodigiosus,* and the W.C.'s on the east side were left in their natural condition. The result was that colonies of the special organism appeared in the plates placed on the west of the hospital, but none were found on the east side.

In all the above experiments the plates were left in the chambers and soil pipes for 24 hours, consequently the *B. prodigiosus* might have resulted from dried particles of the growth separated from the walls of the pipes and chambers and carried upwards by air currents, as well as from the bursting of bubbles and ejection of droplets from the flowing sewage. In order to determine whether the separation of dried particles was the source from which the bacteria were obtained, the experiments were repeated, but on these occasions the plates were withdrawn as soon as the W.C.'s had been flushed, each experiment only taking 20 minutes. When incubated the plates showed practically as many colonies of the *B. prodigiosus* as in the previous experiments, showing that special bacteria may appear in the air of the chambers and pipes independently of the separation of dried particles.

It might be objected that in the experiments just related the bacteria were suspended in water and had no organic matter adhering to them such as would be the case under natural conditions. When fæcal material is flushed down W.C.'s and carried through a drainage system, it is probable that the *B. coli* and the *B. typhosus* in the case of typhoid stools, will have an organic envelope which may materially affect the results.

In order to ascertain whether the objection raised had any force, plates of litmus-lactose-nutrose-agar were suspended in the ventilating pipe of the Centre Block for 24 hours and then incubated. All colonies having a reddish tint were fished and carefully studied, the result being that typical members of the coli group were readily isolated. Plates were also suspended in large inspection chambers connected with one of the main sewers of the town, about 10 feet above the flowing sewage. The plates were removed at the end of

4 hours and incubated, when typical members of the coli group were again readily isolated. As a final test, the possibility of the *B. typhosus* being ejected from typhoid stools was ascertained by using the apparatus shown in fig. 7. The trap was filled with sewage, and litmus-lactose-nutrose-agar plates, the media facing upwards, were suspended in the vertical pipe, which was afterwards covered with a glass plate. A typical stool, obtained from a case of enteric fever under treatment in the military hospital, was then mixed with 2 gallons of water placed in the can connected by indiarubber piping, 1·5 inches in diameter, with the house side of the trap. The taps were next opened, and the contents of the can having passed slowly through the trap, were collected in the can connected to the **P** outgo of the trap. The taps were then turned off and, the cans having been changed, the infected sewage was again passed through the trap. This procedure was followed until the sewage had passed 12 times through the trap. The plates were then removed and incubated. Next day several transparent, blue colonies were observed these were fished and planted on agar slopes. The growths resulting were tested with an anti-typhoid horse serum, and one was found to be completely agglutinated by the serum diluted 1—500. The growth was then submitted to the usual tests, which showed that under the conditions mentioned a true *B. typhosus* had been carried up the vertical pipe.

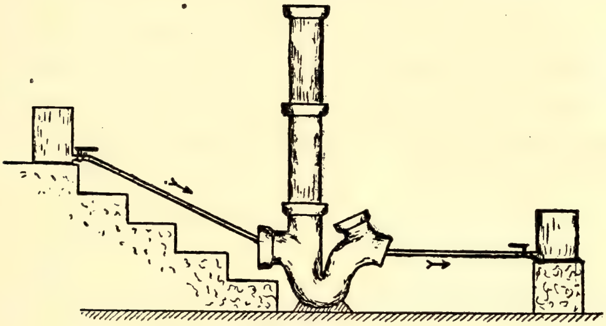


FIG. 7.

Two days later the experiment was repeated with another stool from the same patient, with the result that three colonies of *B. typhosus* were isolated, one being in a plate 2 feet above the trap and the other two in a plate 3 feet 6 inches above the trap.

The experiments were so conducted that no splashing could possibly occur, and on looking through the glass plate on the top of the vertical pipe, when sewage was flowing through the trap, a few bubbles were seen bursting at the surface of the fluid. The pipes and trap employed were quite new, and had not been used in any of the previous experiments.

These results show that bacteria existing in sewage under natural conditions can be ejected into the air in the same manner as the naked bacteria used in the experiments already recorded.

Conclusions.

The experiments show that :—

(1) Specific bacteria present in sewage may be ejected into the air of ventilation pipes, inspection chambers, drains, and sewers by (*a*) the bursting of bubbles at the surface of the sewage, (*b*) the separation of dried particles from the walls of pipes, chambers, and sewers, and probably by (*c*) the ejection of minute droplets from flowing sewage.

(2) A disconnecting trap undoubtedly prevents the passage of bacteria, present in the air of a sewer, into the house drainage system.

(3) An air inlet, even when provided with a mica valve, may be a source of danger when it is placed at or about the ground level.

On the Pathology of the Dropsy produced by Obstruction of the Superior and Inferior Venæ Cavæ and the Portal Vein.—Preliminary Communication.

By Charles Bolton, M.D., Assistant Physician to University College Hospital.

(Communicated by Professor Sidney Martin, F.R.S. Received December 19, 1906,—Read January 17, 1907.)

(From the Pathological Laboratory, University College.)

In August, 1903, I published a paper in the 'Journal of Pathology' (1), in which I demonstrated a method of experimentally producing uncompensated heart disease in an animal, which was compatible with life. This method consisted in diminishing the size of the pericardial sac by stitches, so that the diastolic filling of the heart was impeded. The main symptoms of this condition were dropsy and diminution in the amount of urine excreted.

As the immediate result of this interference with the action of the heart, there occurred a rise of pressure throughout the whole systemic venous system extending as far back as the capillaries, and a fall of the mean arterial blood-pressure. Further, I found that the pressure in all the veins fell to the normal limit again within the space of about one hour, and that subsequently, when dropsy was being produced, the venous pressure in all parts of the body was normal, and the arterial pressure had almost recovered itself.

I wish to draw special attention to the fact that the venous pressure was at first raised not only in the great veins near the heart, but also in the head, limbs, and intestines, that it very rapidly reached the normal limit again, and that the dropsy was produced whilst the venous pressure was normal, the arterial pressure very little if at all lowered, and the capillary pressure therefore not raised.

My reason for so doing is because Starling's (2) theory of hydræmic plethora is founded upon the assumption that the venous pressure primarily falls below the normal in the limbs and intestines, although it rises in the great veins near the heart. The low venous pressure in the limbs and intestines, according to Starling, initiates the absorption of lymph, which continues until the capillary pressure in the limbs as well as in the trunk is raised above normal by the hydræmic plethora so produced. Dropsy is then brought about by the *raised capillary pressure.* He also considers that there must be an alteration in the capillary wall, and he lays great stress upon this point.

In order to examine the immediate effects upon the circulation of the blood, Starling produced his uncompensated condition of the circulation by introducing oil into the pericardium, which brings about precisely the same immediate results as any other condition interfering with the diastolic filling of the heart. He, however, registered the venous pressure in the limb by means of a plethysmograph instead of by the direct introduction of a cannula into a vein of the foot. The result was that he obtained a contraction of the limb, which he interpreted as indicating a fall of venous pressure. I have already pointed out in my paper that this contraction was an indication of the vaso-motor constriction, which occurs as the result of asphyxia of the vaso-motor centre. If the venous pressure be taken by means of a cannula inserted into a vein of the foot, it is quite easy to demonstrate at once a rise of venous pressure in that part, whether the pericardium be filled with oil (see Ref. 1, experiment) or constricted by means of stitches (Ref. 1), or whether the inferior vena cava be obstructed (see Ref. 1, experiment). I, moreover, showed that it was impossible to keep up the high venous pressure by the injection of salt solution so as to produce hydræmic plethora. This is owing to the ready distensibility of the veins and the increased activity of the excretory glands.

The results of my experiments were, therefore, antagonistic to Starling's theory of hydræmic plethora, and I maintained that passive œdema was primarily due to *malnutrition of the capillary wall*, raised capillary pressure playing no actual part in its production.

The present investigation was undertaken from three points of view:—(1) To confirm my previous results by employing a different method. (2) To discover a more easy method of obstructing the venous flow into the heart, and one which admitted of different degrees of obstruction, which could be accurately measured. (3) To bring the results of general venous obstruction into line with those of local venous obstruction, in order to discover the common principle underlying both.

The subject will be treated of under the following headings:—

1. Method.

2. Effects of obstruction of the superior vena cava:—

    (*a*) Upon the production of dropsy.
    (*b*) Upon the circulation of the blood.

3. Effects of obstruction of the inferior vena cava:—

    (*a*) Upon the production of dropsy.
    (*b*) Upon the circulation of the blood.

4. Effects of obstruction of the portal vein :—
    (*a*) Upon the production of dropsy.
    (*b*) Upon the circulation of the blood.
5. Effects of obstruction of the superior, together with the inferior, vena cava :—
    (*a*) Upon the production of dropsy.
    (*b*) Upon the circulation of the blood.
6. Production of hydræmic plethora after obstruction of the inferior vena cava.
7. Conclusions.

1. METHOD.

The animals used were cats. The anæsthetic in all cases was ether, and morphia was administered in addition in those experiments relating to the effects upon the blood-pressures.

In order to obtain access to the superior and inferior venæ cavæ, the chest was opened by an incision an inch or more long and parallel to the ribs in the third right intercostal space in the case of the superior, and in the seventh right space in the case of the inferior vena cava. The ribs were drawn apart by weighted retractors and the lung held aside by a small copper spatula.

If the vein were to be completely obstructed, it was ligatured with silk ; if incompletely obstructed, a piece of Jacques' rubber catheter of the appropriate diameter and about $\frac{1}{4}$ inch in length was placed round the vein and tied on.

In those experiments, which were done in order to determine, by a gradually increasing constriction, at what point the venous or arterial pressure was first altered, a fine wire was passed round the vein and the free ends of this wire pushed through a piece of glass tubing so as to form a snare. The loop so formed was gradually tightened until an effect was observed upon the blood-pressure ; the vein was then cut through and the wire loop removed and its diameter measured.

In all cases during the operation, artificial respiration was maintained through a tracheotomy tube if the animal were to be killed at the end of the experiment ; but if it were to be allowed to live, a piece of glass tubing was introduced into the larynx through the mouth and artificial respiration maintained in this way, the air being blown through a bottle containing ether. Strict antiseptic precautions were observed and a *post-mortem* bacteriological examination made in most of the cases.

No difficulty with regard to pneumothorax was experienced after the chest was stitched up, and the animals began to breathe naturally at once.

The arterial blood-pressure was registered by means of a mercurial manometer, the cannula being placed in either the carotid or femoral artery, and a float, which traced the pressure curve on a kymograph.

The venous pressures were taken in the inferior vena cava, the cannula being introduced into the external iliac vein; at the lower end of the femoral vein where it divides into two branches at the ankle, the cannula being inserted into one of the branches and pushed on until its point was flush with the opening into the femoral vein; in the portal vein, the cannula being introduced into a branch of the splenic vein, the spleen being then placed back in the abdomen; at the upper end of the external jugular vein, the cannula being inserted into the posterior auricular vein and pushed down until its point was flush with the opening into the jugular vein. The venous pressure was registered on the kymograph by means of an air tambour connected with the top of the venous manometer by means of rubber tubing. This simply demonstrates whether a rise or fall of venous pressure occurs: it is no indication of the exact amount of such rise or fall. The diameter of the inferior vena cava is 5 mm., that of the superior vena cava practically the same or a trifle smaller, and that of the portal vein 4 mm.

2. Effects of Obstruction of the Superior Vena Cava.

(a) *Upon the Production of Dropsy.*

The vein was obstructed both above and below the entrance of the azygos vein (*i.e.*, between the entrances of the internal mammary and azygos veins, and between the azygos vein and the right auricle).

Obstruction below the Azygos Vein.

Complete Occlusion.—In each case death resulted either on the day following the operation or five or six days later. The *post-mortem* findings were the same in all cases. There was marked œdema of the mediastinum, extending up into the neck and down along the inferior vena cava to the diaphragm, the whole of the cellular tissue being distended with clear serous fluid. A slight amount of clear fluid was found in the pericardium, and bilateral hydrothorax was present. In one case 25 c.c. serous fluid was found in each pleural cavity; sometimes this fluid is blood stained. The œdema of the mediastinum may extend through the diaphragm, and out as far as the axillæ. There is no free fluid in the abdomen. Œdema at the seat of the wound occurs in the subcutaneous and muscular tissues, but this is not continuous with the mediastinal œdema and is dependent upon mechanical laceration of the tissues. The urine is diminished in amount, the animal refuses food, and wastes.

Obstruction above the Azygos Vein.

Complete Occlusion.—I have been able to show that an animal may completely survive this operation. The animal lived for three months, and at the *post-mortem* examination the vein was absolutely occluded and not a trace of œdema to be seen. The anastomoses were established by the comes nervi phrenici, the internal mammary, and the azygos veins chiefly; the veins of the diaphragm were also distended.

Before these anastomoses are established, however, œdema is regularly produced, and apparently may directly lead to the death of the animal, or absolutely disappear as in the above case. This can be proved by killing the animal on the day after the operation or at a later date. Œdema of the mediastinum, slight hydropericardium, and bilateral hydrothorax are found. It seems to be the exception for all the fluid to be absorbed. One animal died 18 days after the operation in an attack of dyspnœa, and 145 c.c. slightly blood-stained fluid was found in the right pleural cavity and 125 c.c. in the left; œdema of the mediastinum was also present. I have never found any free fluid in the peritoneal cavity. The urine is diminished in amount.

Incomplete Occlusion. Diameter of Tube, 1½ *mm.*—The result of constriction to this size is absolutely the same as that of complete ligature. As a fact, the tube practically obliterates the vein, since water cannot be made to flow through it *post mortem.* At any rate, if not absolutely obliterated, thrombosis no doubt completes the obstruction.

Diameter of Tube, 2 *mm.* Œdema of the mediastinum and hydrothorax are produced in this case also. Fluid flows slowly through the constriction *post mortem.*

Diameter of Tube, 3 *mm.*—Œdema of the mediastinum is produced with this constriction also, but only the merest trace of fluid in the pleural cavity is found. Fluid flows quite easily through the constriction *post mortem.*

Diameter of Tube, 4 *mm.* No œdema is produced by this amount of constriction of the superior vena cava. There is slight hæmorrhage and œdema in the subcutaneous tissues at the seat of the wound, but this, as in all the other cases, is the direct result of the injury to the tissues.

The wall of the vein is so very thin that its diameter is practically the same as that of the enclosing tube in the case of 3- and 4-mm. constrictions. The general statement may therefore be made that when the diameter of the superior vena cava is constricted to three-fifths of its normal size, œdema of the mediastinum and hydrothorax begin to appear, and that it is possible, but not usual, for an animal to absorb the fluid and completely recover when the superior vena cava is absolutely obliterated. This statement applies to constrictions which are suddenly produced.

(b) *Upon the Circulation of the Blood.*

Arterial Pressure.

Complete ligature of the superior vena cava produces only a very slight effect upon the arterial blood-pressure curve. The output of the heart is slightly diminished, and there may be a very trifling fall in the pressure, but usually there is none. On the following day, *when the animal has developed dropsy, the blood-pressure is of precisely the same height as it was in the normal animal.* The fact that there is no immediate fall in the blood-pressure on complete ligature of the superior vena cava was, of course, well known in the days of Cohnheim (3).

Constriction of the superior vena cava similarly brings about no alteration in the height of the blood-pressure tracing.

Venous Pressure.

External Jugular Vein.—A *gradually increasing constriction* of the superior vena cava produces no change whatever upon the pressure at the upper end of the external jugular vein, until the former vein is narrowed to a diameter of 3 or $2\frac{1}{2}$ mm. At this point the pressure in the jugular vein rises to a height varying from 20 to 40 mm. $MgSO_4$ solution above what it was before the constriction was commenced.

Complete ligature causes a rise of pressure at the upper end of the external jugular vein of about 130 mm. $MgSO_4$ solution above the normal. This high pressure is not maintained, for, whether the superior vena cava has been completely ligatured or merely constricted, the pressure in the jugular vein gradually falls, and *within the space of about an hour has reached the normal limit once more.* On the following day, *when the animal has developed dropsy, the venous pressure in the external jugular vein is still normal.*

The following experiment shows the effects of obstruction of the superior vena cava upon the pressure at the upper end of the external jugular vein:—

Oct. 10, 1906. Cat; weight, 2280 grammes. Morphia, $\frac{1}{7}$ grain. Ether and artificial respiration.

Pressures.	Femoral artery.	External jugular vein.
Before obstruction	60 mm. Hg	100 mm. $HgSO_4$ solution
Obstruction to 2 mm.	60 „	111 „ „
1 minute later	—	112 „ „
1 „ „	—	112 „ „
1 „ „	66 mm. Hg	113 „ „
Complete obstruction	54 „	205 „ „
5 minutes later.....................	—	221 „ „
5 „ „	—	206 „ „
5 „ „	—	185 „ „
5 „ „	—	174 „ „
5 „ „	—	170 „ „
5 „ „	60 mm. Hg	130 „ „
5 „ „	—	110 „ „
5 „ „	—	105 „ „

Inferior Vena Cava.—On complete ligature of the superior vena cava there is a very trifling and temporary fall of about 10 or 15 mm. $MgSO_4$ solution in the pressure in the inferior vena cava.

Portal Vein.—Complete occlusion of the superior vena cava or constriction of its diameter to 2 mm. causes a very transitory fall of pressure of about 5 mm. $MgSO_4$ solution in the portal vein.

The only significance of this slight fall of pressure in the portal vein and inferior vena cava is that it indicates the abstraction of blood from these venous territories and its retention in that of the superior vena cava.

It is quite apparent, therefore, that the smallest degree of constriction of the superior vena cava, which is necessary to produce any effect upon the venous pressure, will give rise to dropsy.

3. EFFECTS OF OBSTRUCTION OF THE INFERIOR VENA CAVA.

(a) *Upon the Production of Dropsy.*

In all cases the vein was obstructed just below the heart, between it and the diaphragm.

Complete Occlusion.—Complete ligature of the inferior vena cava above the diaphragm leads to death in a few hours. There is regularly found 15 c.c. or more blood-stained fluid in the peritoneal cavity. No œdema is found anywhere else. This is exactly the result obtained by Richard Lower (4), more than 200 years ago, by ligature of the inferior vena cava in the chest, although it is usually misquoted in the text books, which state that he obtained œdema of the hind legs. Antiseptics were not recognised in his day and, so far as I am aware, he left the lung collapsed. In fact, it was precisely this collapse of the lung which induced Ranvier (5) to ligature the vein in the abdomen, thinking that if dropsy were to be produced by venous obstruction he should obtain it by this means; however, in this case the anastomoses are too readily established, and Ranvier fell into error in thinking he had disproved Lower's results.

Incomplete Occlusion. Diameter of Tube, 1½ *mm.*—This practically amounts to total occlusion of the vein, and death results with about 20 c.c. fluid in the peritoneal cavity.

Diameter of Tubes, 2 *and* 2½ *mm.*—Constrictions to both these diameters may lead to death in a few hours, with 15 to 20 c.c. blood-stained fluid in the peritoneal cavity; on the other hand, the animal may survive the operation and develop ascites, but for how long I have not determined.

Diameter of Tube, 3 *mm.*—The animal survives, but if killed 24 hours after the operation, 25 to 35 c.c. clear straw-coloured fluid will be found in the peritoneal cavity, and possibly a few drops of fluid in the pleuræ. One animal lived four months, and at the autopsy showed no dropsy at all. Anastomoses were well established in the abdominal wall, carrying the blood to the superior vena cava. I have found ascites present 13 days after the operation in one case.

The fluid, therefore, is absorbed when the anastomoses are established. The urine is diminished in amount when dropsy is being produced.

Diameter of Tube, 4 *mm.*—A constriction to this amount does not lead to ascites.

The same general statement which was made with regard to constriction of the superior vena cava may be made in the case of the inferior vena cava. When the diameter of this vein is constricted to three-fifths of its normal size, ascites appears, and when the anastomoses are completely established this fluid disappears. If the vein is constricted below two-fifths of its diameter, or if it is completely ligatured, death will result in a few hours, and some ascitic fluid will be found in the peritoneal cavity.

This statement, of course, applies to suddenly produced constrictions.

(b) *Upon the Circulation of the Blood.*

Arterial Pressure.

A gradually increasing constriction of the inferior vena cava in the chest is perfectly compensated for until a diameter of 3 mm. is reached, when the arterial blood-pressure falls about 20 mm. Hg. Constrictions greater than this produce proportionately greater depressions in the blood-pressure curve, and *complete ligature* of the inferior vena cava causes the arterial pressure to fall to 30 or 40 mm. Hg above zero.

It has for many years been known that ligature of either the inferior vena cava or the portal vein causes a great fall in the arterial pressure owing to accumulation of blood in the splanchnic area (3).

On the following day, when ascites is present, the arterial blood-pressure is usually below what it was before the operation, if the animal survives.

Venous Pressure.

Inferior Cava.—A gradually increasing constriction of the inferior vena cava in the chest produces no rise of pressure at the lower end of that vein until its diameter has been reduced to 3 mm., when the pressure rises about 20 to 30 mm. $MgSO_4$ solution.

Constriction to a diameter of 2 mm. produces a rise of about 50 mm. $MgSO_4$ solution, and complete ligature a rise of 100 mm. or more. Whatever be the degree of constriction, however, *the pressure in the inferior vena cava gradually falls* and after an hour or longer has reached the normal.

Moreover, on the following day, *when the animal has developed ascites, the pressure in the inferior vena cava still remains normal.*

The following experiment shows the relative effects of constriction of the inferior vena cava in the chest to a diameter of $2\frac{1}{2}$ mm., and of complete ligature. The arterial pressure was taken in the carotid artery and the venous pressure in the external iliac vein.

Sept. 22, 1906. Cat; weight, 2600 grammes. Morphia, $\frac{1}{7}$ grain. Ether and artificial respiration.

Pressures.	Carotid artery.	External iliac vein.
Before obstruction	90 mm. Hg	95 mm. $MgSO_4$ solution
Obstruction to $2\frac{1}{2}$ mm.	66 ,,	115 ,, ,,
8 minutes later	82 ,,	130 ,, ,,
Vein completely occluded	30 ,,	210 ,, ,,
2 minutes later	30 ,,	215 ,, ,,

Veins of Foot.—Complete ligature of the inferior vena cava in the chest produces a rise of pressure in the veins of the foot of 60 mm. $MgSO_4$ solution or more. Constriction to a diameter of 2 mm. may cause a rise of 50 mm. $MgSO_4$ solution in the veins of the foot. This is a more gradual rise than that in the inferior vena cava, because it is produced in veins farther removed from the seat of obstruction.

The pressure in these veins falls to the normal limit again in about one hour after the occlusion has been produced.

The following experiment shows the effects of obstruction of the inferior vena cava in the chest upon the venous pressure in the foot.

Oct. 24, 1906. Cat; weight, 3200 grammes. Morphia, $\frac{1}{6}$ grain. Ether and artificial respiration.

Pressures.	Carotid artery.	Vein of foot.
Before obstruction	110 mm. Hg	142 mm. MgSO$_4$ solution
After complete obstruction	46 "	204 " "
Obstruction removed	104 "	120 " "
2 minutes later	110 "	135 " "
Obstruction to 2 mm.	64 "	160 " "
5 minutes later	—	195 " "
5 " "	96 mm. Hg	191 " "
5 " "	—	193 " "
5 " "	—	183 " "
5 " "	—	184 " "
5 " "	—	183 " "
5 " "	—	185 " "
5 " "	—	172 " "
5 " "	70 mm. Hg	165 " "
5 " "	—	164 " "
5 " "	56 mm. Hg	158 " "
5 " "	—	152 " "
5 " "	—	143 " "
Obstruction removed	74 mm. Hg	123 " "
3 minutes later	80 "	120 " "

The following experiment demonstrates the fact that the pressure in the veins of the foot rises when the pressure in the pericardium is increased by the introduction of oil into that sac.

Nov. 24, 1906. Cat; weight, 3050 grammes. Morphia, $\frac{1}{6}$ grain. Ether and artificial respiration.

Pressures.	Carotid artery.	Vein of foot.
Before introduction of oil	84 mm. Hg	120 mm. MgSo$_4$ solution
		135 " "
		142 " "
During injection, $\frac{1}{2}$ minute intervals	—	145 " "
		148 " "
Injection stopped	48 mm. Hg	152 " "
Oil allowed to run out	—	148 " "
Pressures at $\frac{1}{2}$ minute intervals	—	141 " "
		135 " "
		135 " "
	90 mm. Hg	132 " "
		132 " "
	84 "	130 " "
Oil introduced 2nd time	—	135 " "
Pressures at $\frac{1}{2}$-minute intervals	—	143 " "
		148 " "
	32 mm. Hg	150 " "
Oil allowed to run out	—	138 " "
Pressures at $\frac{1}{2}$-minute intervals	—	135 " "
		130 " "
		126 " "
		124 " "
	90 mm. Hg	115 " "

Portal Vein.—Whether the inferior vena cava be completely occluded or constricted to 2 mm. in diameter, the pressure in the portal vein rises, in the former case about 90 mm., in the latter 50 to 60 mm. $MgSO_4$ solution. The pressure falls to the normal limit again in about the same time as it does in the case of the inferior vena cava. On the following day, *when ascites is present, the pressure in the portal vein is still normal.*

The following experiment shows the effects of obstruction of the inferior vena cava in the chest upon the pressure in the splenic vein.

Oct. 1, 1906. Cat; weight, 2535 grammes. Morphia, $\frac{1}{7}$ grain. Ether and artificial respiration.

Pressures.	Carotid artery.	Splenic vein.
Before obstruction	120 mm. Hg	113 mm. $MgSO_4$ solution
After complete obstruction	60 ,,	200 ,, ,,
2 minutes later	44 ,,	175 ,, ,,
2 ,, ,,	—	133 ,, ,,
1 ,, ,,	—	115 ,, ,,
Obstruction removed	80 mm. Hg	77 ,, ,,
1 minute later	—	90 ,, ,,
1 ,, ,,	—	114 ,, ,,
½ ,, ,,	90 mm. Hg	113 ,, ,,
Complete obstruction, 2nd time	42 ,,	170 ,, ,,
2 minutes later	—	177 ,, ,,
2 ,, ,,	—	135 ,, ,,
1 ,, ,,	—	115 ,, ,,
Obstruction removed	88 mm. Hg	85 ,, ,,
2 minutes later	86 ,,	120 ,, ,,
1 ,, ,,	—	113 ,, ,,

External Jugular Vein.—When the inferior vena cava is obstructed to 2 mm., the pressure at the upper end of the external jugular vein falls to the trifling extent of about 10 or 15 mm. $MgSO_4$ solution. This fall indicates an abstraction of blood from the territory of the superior vena cava, on account of the accumulation of blood in the portal area.

The following experiment shows that on obstructing the inferior vena cava in the chest the pressure in the external jugular vein falls:—

Oct. 18, 1906. Cat; weight, 2750 grammes. Morphia, $\frac{1}{7}$ grain. Ether and artificial respiration.

Pressures.	Carotid artery.	External jugular vein.
Before obstruction	74 mm. Hg	56 mm. $MgSO_4$ solution
Directly after complete obstruction	32 ,,	25 ,, ,,
Obstruction removed	70 ,,	56 ,, ,,

The smallest constriction, therefore, of the inferior vena cava, which is necessary to produce any effect upon the blood-pressures, will give rise to ascites.

4. EFFECTS OF OBSTRUCTION OF THE PORTAL VEIN.

(a) *Upon the Production of Dropsy.*

Complete Occlusion.—Ligature of the portal vein at the spot where it enters the liver brings about death in a variable number of hours. There is regularly found about 6 or 10 c.c. slightly blood-stained fluid in the peritoneal cavity and small hæmorrhages in the mesentery may be seen. Since ligature of the superior mesenteric vein causes hæmorrhagic infarction of the intestine, whilst ligature of the portal vein does not, the latter fact is to be explained by the circumstance that the veins from the stomach and spleen are included in the ligature, and, therefore, a certain amount of venous anastomosis can become established and the spleen itself can act as a reservoir, but the anastomoses are not sufficient to prevent the occurrence of dropsy or the great fall of arterial blood-pressure which brings about the death of the animal.

Incomplete Occlusion. Diameter of Tube, 1½ *mm.*—Constriction of the portal vein down to 1½ mm. diameter may or may not cause the death of the animal.

If the animal dies, about the same amount of free ascitic fluid is found in the peritoneal cavity as when the portal vein is completely ligatured. If the animal lives and is killed on the following day, 20 c.c. fluid, free from blood, may be found in the peritoneal cavity.

This is precisely what would be expected, since the animal lives longer than when the vein is completely occluded, the obstruction not being so great.

Diameter of Tube, 3 *mm.*—Constriction to a diameter of 3 mm. likewise produces ascites in small amount, about 10 c.c. perfectly clear fluid being found in the peritoneum on the day after the operation. This fluid, however, may be absorbed, because in an animal whose portal vein had been constricted to this diameter seven weeks previously no ascites was found. The fluid may also be absorbed when the portal vein is constricted to 2 mm. in diameter.

Diameter of Tube, 4 *mm.*—In most large animals the diameter of the portal vein is just over 4 mm. in size, and a slight but definite constriction is produced by a tube of 4 mm. in diameter. Ascites, however, is not produced by such a procedure, the abdominal cavity being perfectly normal.

Speaking broadly, therefore, constriction of the portal vein to about three-fourths of its normal diameter will give rise to dropsy of the peritoneum. Complete ligature will only produce a small amount of dropsy of the peritoneum and small hæmorrhages into the mesentery.

It is probable that constriction of the inferior vena cava must be carried to a rather greater relative extent than that of the portal vein to produce dropsy, because the anastomoses are more readily established in the case of the former than in that of the latter.

(b) *Upon the Circulation of the Blood.*

The effects of obstruction of the portal vein, as is well known, are a marked fall of arterial blood-pressure (3) and a rise of venous pressure in the splanchnic territory.

After complete ligature of the portal vein I have found that the venous pressure in the territory of this vein, although it rises enormously behind the ligature, *falls to the normal limit again,* as in the case of the inferior and superior venæ cavæ, and the small amount of free fluid found in the peritoneum is formed whilst the portal pressure is normal. Small hæmorrhages may be produced whilst the portal pressure is raised, but the dropsy is produced after the venous pressure has reached its normal limit.

After constriction of the portal vein to 2 mm. diameter, dropsy of the peritoneum is produced within 24 hours, but this may subsequently disappear and complete compensation be established, the arterial and venous pressures being normal.

5. EFFECTS OF OBSTRUCTION OF THE SUPERIOR, TOGETHER WITH THE INFERIOR, VENA CAVA.

(a) *Upon the Production of Dropsy.*

The superior vena cava was constricted, and after an interval of 14 or 20 days the inferior cava was constricted, or, *vice versâ,* the superior vena cava was constricted in an animal whose inferior vena cava had been constricted 14 or 20 days previously. In some cases the azygos vein was ligatured at the same time as the superior vena cava was constricted.

The *post-mortem* findings, both the superior and inferior cavæ being constricted, are exactly the same as I described in my former paper as resulting from constriction of the pericardium, in other words, the condition is exactly comparable to that resulting from an uncompensated heart lesion.

Dropsical fluid is found in each pleural cavity, in the peritoneal cavity, and in the mediastinum.

If the inferior cava be constricted to a diameter of 4 mm., the superior to that of 3 mm., and the azygos vein be ligatured, the animal may survive, and a month later no fluid whatever be found *post mortem.*

Dropsy of the pleuræ and peritoneum is regularly found if the inferior cava be constricted to a diameter of 3 mm., the superior to that of 3 mm., and the azygos vein be ligatured.

This is a more easy method of producing uncompensated heart disease in an animal than is constriction of the pericardium, because the exact amount of constriction is known at the time of operation, and it does not depend upon the judgment of the operator, so that there is less fear of the animal dying or of the operation being unsuccessful.

(b) *Upon the Circulation of the Blood.*

In these experiments the inferior vena cava was constricted to a diameter of 3 mm., and the superior vena cava was completely ligatured.

The same results are obtained whether the azygos be ligatured or not.

The effects upon the arterial and venous pressures are exactly the same as those produced when the pericardium is constricted, with the exception that the rise of venous pressure is most marked in the external jugular vein, because the superior vena cava is completely obstructed.

The arterial pressure falls considerably, and the venous pressure in all parts of the body rises. The venous pressure falls to the normal level again in about the same time as it does when either vein is constricted separately, and *dropsy is produced whilst the venous pressure is normal.*

The effects of obstruction of both superior and inferior venæ cavæ are, therefore, precisely the same as those produced by an uncompensated heart lesion.

6. PRODUCTION OF HYDRÆMIC PLETHORA AFTER OBSTRUCTION OF THE INFERIOR VENA CAVA.

If the inferior vena cava be constricted, and if, after the pressure in the external iliac vein has fallen to its normal level subsequently, salt solution be very slowly injected into the external jugular vein in quantities of 5 c.c. at a time, a large amount may be introduced without raising the venous pressure behind the constriction or the arterial pressure. If during the injection the venous pressure rises 10 mm. $MgSO_4$ solution (less than a pressure of 1 mm. Hg), it quickly falls again after the injection. In this way, during one and a-half hours I have injected 130 c.c., and still the pressures, arterial and venous, remained normal. Absorption of lymph occurs much more slowly than this, and the composition of lymph is different from that of salt solution. It is therefore inconceivable to me how an animal by absorption can raise its capillary pressure by the production of hydræmic plethora. In the case in point 10 c.c. ascitic fluid was found in the peritoneal cavity at the end of the injections, and this fluid was produced with a normal capillary pressure.

If such a procedure be carried out in the case of a normal animal, no ascites is found after intravenous injection of 130 c.c. fluid.

7. CONCLUSIONS.

Of whatever nature the impediment be which opposes the free flow of blood into the heart and diminishes thereby the diastolic blood quantum, it will bring about precisely the same effects upon the circulation of the blood. There will be a general rise of systemic pressure affecting all the veins of the body and extending back as far as the capillaries, and a fall of the mean arterial blood-pressure. This effect is due to accumulation of blood in the veins. The venous pressure, however, soon falls to its normal level again, if the animal lives; and the arterial pressure is raised more or less completely to its former level owing to vaso-motor constriction. Dropsy is produced after several hours, whilst the capillary pressure is normal or even below normal.

The reason why the venous pressure returns to the normal level is because the veins passively distend and likewise the capillaries, which normally are not completely filled. The veins which distend are those of the thorax and abdomen, because the superior and inferior cavæ can be seen distended and the liver enlarged; the venæ cavæ are less supported than those in other parts of the body, they are situated within the suction action of the thorax, the bed of the blood stream is here smaller than in the more peripheral parts of the body, and muscular tension empties the veins in the latter situation towards the heart. In the erect posture, when gravity is of the greatest importance, the blood will tend to collect in the most dependent parts.

The dropsy is produced in this situation where the veins and capillaries are distended and where the blood flows with a diminished velocity, and it probably *depends entirely upon an altered condition of the vessel wall*, and not upon an altered condition of the nutrition of the tissues themselves, as maintained by Lazarus-Barlow (6), although, of course, these experiments do not disprove Barlow's theory.

This alteration is probably of a pathological nature and not such as would lead to an increase of the normal function of the capillary endothelium (7). *Raised capillary pressure plays no part in the production of this œdema, and the arterial pressure may be normal or less than normal, it matters not which.*

It might be argued that during the first hour the raised pressure which occurs would damage the vessel wall, but in those animals which recover and develop dropsy, and in which the constriction is not very complete, the raised pressure is relatively insignificant and not to be compared with that occurring during muscular movement. The earliest dropsy, therefore, in uncompensated heart disease is strictly local in origin, and the capillaries of the district affected become, practically speaking, an excretory organ. In the remaining parts of the body absorption must occur *pari passu* with this increased output of fluid, in order to keep up the normal amount of blood in the body, but in the part affected absorption by the veins must be retarded.

As the disease becomes more extensive the area of production of the dropsy must extend.

When the superior or inferior vena cava is obstructed alone, the same initial rise of pressure occurs behind the obstruction as far back as the capillaries, and it soon falls to the normal level again, owing to distension of the particular veins in question and also, to some extent, on account of the anastomotic channels, which after many days may effect complete compensation if the constriction is not too complete. The dropsy is produced in exactly the same way as described above, when *all* the venous channels opening into the heart are obstructed, and it disappears in those cases in which complete compensation is subsequently effected by the anastomoses.

Absolutely the same remarks apply to obstruction of the portal vein.

Are there any conditions in which the dropsy of venous stagnation is produced when the capillary pressure is high?

In answer to this question it may be said that the necessary conditions are: *Firstly,* that the venous territory in which the obstruction is situated be not too large in proportion to the rest of the vascular territories of the body, so that sufficient blood can be spared to maintain a high venous pressure, and, *secondly,* that the anastomoses be not too free. Under these circumstances the dropsy of venous stagnation will occur with a high capillary pressure, but the actual cause of the œdema is, however, an alteration in the capillary wall, the high pressure being merely a concomitant circumstance, or at the most a contributing factor. A parallel instance is seen in the case of hæmorrhagic infarction. The escape of blood from the vessels in hæmorrhagic infarction may occur when the capillary pressure is high, as in venous obstruction, but it may also occur when the capillary pressure is low, as in arterial obstruction.

The dropsy of *local* venous stagnation is, therefore, produced in exactly the same manner as that resulting from *general* venous stagnation, the common factor underlying both being an altered condition of the vessel wall.

In the former case the capillary pressure may be high, but not so in the latter case. To maintain the volume of the blood normal in both these conditions, absorption must occur in the unaffected portions of the vascula system, but this absorption is not so great as it would be if the excretion of urine were not diminished. The dropsy of general venous stagnation is essentially local in origin, and the locality increases in extent as the disease advances.

Experiments are now being undertaken to estimate the lymph flow in the various pathological conditions considered above.

REFERENCES.

1. Bolton, "The Experimental Production of uncompensated Heart Disease, with Especial Reference to the Pathology of Dropsy," 'Journ. of Path. and Bact., August, 1903, p. 67.
2. Starling, "Some Points in the Pathology of Heart Disease" (Arris and Gale Lectures), 'Lancet,' London, 1897, vol. 1, pp. 569—723. "Physiological Factors involved in the Causation of Dropsy" (Arris and Gale Lectures), 'Lancet,' London, 1896, vol. 1, pp. 1267—1407. 'Practitioner,' Nov. and Dec., 1905, pp. 633—786 (quoted by Bainbridge). 'Lancet,' Sept. 29, 1906 (quoted by H. Starling).
3. Cohnheim, "Vorlesungen über allg. Pathologie," Berlin, 1882, Aufl. 2, vol. 1 ("Lectures on General Pathology," 'New Syd. Soc. Trans.,' London, 1889, vol. 1, p. 167).
4. Lower, "Tractatus de Corde, item de Motu et Colore Sanguinis," Editio sexta, 1728, p. 127.
5. Ranvier, "Compt. rend. Soc. de Biol.,' Paris, 1869, vol. 59, p. 1326.
6. Lazarus-Barlow, "Œdema," 'Phil. Trans.,' B, 1894, vol. 185, p. 779.
7. Hamburger, "Stauungshydrops und Resorption," 'Virchow's Archiv,' 1895, vol. 141, p. 398.

The Influence of Increased Barometric Pressure on Man. No. 3. *—The Possibility of Oxygen Bubbles being set free in the Body.*

By Leonard Hill, F.R.S., and M. Greenwood, Jun., M.R.C.S., Research Scholar of the British Medical Association.

(Received January 26,—Read February 7, 1907.)

The avidity of the tissues for oxygen is such that this gas has never been regarded as a factor in the production of decompression symptoms with respect to the formation of bubbles.

Bert's (1) analyses of the gas set free in the hearts of dogs rapidly decompressed from 10 atmospheres gave—

	Per cent. CO_2.	Per cent. O_2.	Per cent. N_2.
(1)	15·2	2·0	82·8
(2)	15·9	0	84·1
(3)	20·8	a trace	79·2

v. Schrötter and his colleagues (2) found a higher percentage of oxygen in similar analyses—

	Per cent. CO_2.	Per cent. O_2.	Per cent N_2.
(1) 4 dogs yielded	4·71	15·31	79·98
(2) "	12·45	7·18	80·37

Leonard Hill and C. Ham (3) killed rats by rapid decompression from +10 atmospheres, or more, and, cutting up their bodies under a funnel filled with acidulated water, obtained the following results:—

	Per cent. CO_2.	Per cent. O_2.	Per cent. N_2.
(1)	10·7	2·1	87·2
(2)	16·0	4·0	80·0

Hill and Macleod (4) submitted frogs and toads to 20 atmospheres of oxygen (Brin's oxygen, containing 93—95 per cent. O_2) for five minutes, and rapidly decompressed them. They found that the animals were convulsed and enormously distended with gas. In the case of mice submitted to 10 atmospheres for five minutes and rapidly decompressed, no such distension was observed, but the animals went into tetanic convulsions. These convulsions could be excited by a touch, the mice usually recovering and appearing normal on the following day. Mice killed in the convulsive state showed, here and there, a few bubbles in the vessels, some being found, on microscopic examination, in the central nervous system, distending the capillary sized vessels and compressing the nerve-cells. The convulsions were due to oxygen poisoning, and the bubbles, being formed of oxygen, were eventually absorbed by the tissues. These bubbles supplied the nerve-cells with oxygen until the circulation became re-established, so that no lasting paralysis resulted, as occurs with nitrogen bubbles. It seemed, therefore, probable that, if an animal be kept in oxygen until symptoms of poisoning are well marked and there is danger of death, then the tissues will be unable to combine with the oxygen liberated on sudden decompression, and embolism will result. We decided to settle this question by analyses in the peritoneal cavity, blood-vessels, and subcutaneous tissues.

The technic employed was that described in the paper of Hill and Ham (3), the animal being cut open under a funnel filled with water and inverted over a basin of water. Before being passed under the funnel, the gas is carefully washed out of the fur, and pains are taken to avoid opening the digestive tract or lungs. The results obtained on frogs were as follows:—

(1) Frog exposed to +20 atmos. O_2 during 1 hr. 10 mins. Rapid decompression. Composition of gas obtained from the body,—

$$\begin{cases} O_2 \ldots\ldots & 80 \text{ per cent.} \\ N_2 \ldots\ldots & 20 \quad \text{"} \end{cases}$$

(2) Large Dutch frog. +16 to 20 atmos. O_2 during 35 mins. Rapid decompression.

$$\begin{cases} CO_2 \ldots\ldots & 2{\cdot}2 \text{ per cent.} \\ O_2 \ldots\ldots & 75{\cdot}6 \quad \text{"} \\ N_2 \ldots\ldots & 22{\cdot}2 \quad \text{"} \end{cases}$$

(3) Frog. +20 atmos. O_2 during 33 mins. Rapid decompression. Dead on removal.

$$\begin{cases} CO_2 \ldots\ldots & 2{\cdot}5 \text{ per cent.} \\ O_2 \ldots\ldots & 62{\cdot}0 \quad \text{"} \\ N_2 \ldots\ldots & 35{\cdot}5 \quad \text{"} \end{cases}$$

The saturation time in frogs differs so much from that of the Mammalia that no very direct conclusions could be drawn from these results, and we therefore extended our observations to rats, the following experiments being performed:—

(1) Three rats were exposed to +70 lbs. O_2 for one hour. Two were convulsed in 35 minutes, and at the end of the experiment they were all moribund. One, however, still breathed after decompression, which took 3½ seconds. Analysis of the peritoneal gas yielded:—

$$\begin{cases} O_2 \ldots\ldots & 54{\cdot}5 \text{ per cent.} \\ N_2 \ldots\ldots & 45{\cdot}5 \quad \text{"} \end{cases}$$

(2) Three rats were exposed to +75 lbs. O_2 for three-quarters of an hour. Decompression time, 4 seconds. All three were paretic. The peritoneal gas of one contained—

CO_2	2·2 per cent.
O_2	28·3 „
N_2	69·5 „

(3) Three rats exposed for one hour to +75 lbs. O_2. Decompression in 3 seconds. Two rats were dead, the third moribund. Peritoneal gas—

CO_2	6·7 per cent.
O_2	26·7 „
N_2	66·6 „

(A sample of the chamber air contained only 75 per cent. O_2, showing that it had not been completely washed out at the commencement of the experiment, since the gas in the cylinder used in this experiment contained 87·3 per cent. O_2.)

(4) Experiment, 12.12.06—

11.55 A.M.—Six rats and six mice were placed in the pressure chamber, which was washed out with oxygen and then raised to +75 lbs. O_2.

12.0.—Two of the mice convulsed.

12.5.—One rat exhibits a twitching of limbs, all the mice affected.

12.20.—All the rats affected, one convulsed.

12.35.—Decompression in 4 seconds. Several rats immediately thrown into convulsions.

On opening the chamber, four mice were found to be dead, two others affected, one of them dying in a few minutes. Of the rats, one was dead, two convulsed, three moderately affected. Of these five, three died, one was killed, and the fifth recovered. A sample was collected. One mouse (dead) yielded a good deal of gas, one rat (dead) very little, and one rat (killed for analysis) a considerable amount.

The analysis gave—

CO_2 and O_2	67·9 per cent.
N_2	32·1 „.

These experiments are not sufficiently numerous to permit of very exact statements being made, but they seem to show that free oxygen is liberated during decompression if the animal exhibit symptoms of oxygen poisoning.

The onset of convulsions immediately after decompression, mentioned in the above account, is very suggestive, and may well have been due to the sudden liberation of gas bubbles acting on the already poisoned tissues.

The breathing of pure oxygen has been suggested by v. Schrötter (5) as a means of washing the dissolved nitrogen out of the body before decompression. He suggested that deep-sea divers might take down a small cylinder, wash themselves out for five minutes, and then ascend rapidly without risk.

The experiments of Durig (6) show that, on breathing almost pure oxygen, the body nitrogen is washed out in about five minutes, as shown by the quantity of nitrogen in the expired air sinking in that time to a constant level. L. Hill and C. Ham (7) have pointed out the extreme danger of breathing oxygen for even five minutes at high pressures, and v. Schrötter (8) himself has come to the conclusion that the use of oxygen should be confined to an inhalation immediately after decompression.

The gas set free in the blood on rapid decompression contains 79—87 per cent. N_2. Of the alveolar air, nitrogen forms 79 per cent., so that the difference of tension promoting an escape of bubbles is very small or nil (5—0 per cent.).

If pure oxygen be breathed, the difference of tension will become 79—87 per cent., and this will greatly favour the escape of nitrogen bubbles from the pulmonary vessels (Zuntz). There is little advantage in breathing oxygen during the course of a rapid decompression, because the exchange is between the tissues saturated with nitrogen and the blood. The amount carried away by the blood depends on the respective tensions of nitrogen in the tissues and the blood.

Oxygen inhalation cannot affect this, except in so far as it clears the arterial blood of nitrogen in the lungs. If the pressure falls rapidly the tissues will still be saturated—say, at 5 atmospheres—and hold 5 per cent. of nitrogen, while the blood can only carry, say, 3 per cent., the pressure having fallen to 3 atmospheres. Oxygen can have little or no effect in arresting this decreasing power of the blood to carry nitrogen (v. Schrötter).

The only safe method is slow decompression at a uniform rate when the body has been exposed long enough to become saturated.

REFERENCES.

(1) Bert, 'La Pression Barométrique,' p. 955.
(2) Heller, Mager, and Schrötter, 'Luftdruckerkrankungen,' etc., p. 800.
(3) Hill and Ham, "Phys. Soc. Proc.," 'Journ. Physiol.,' vol. 33.
(4) Hill and Macleod, 'Journ. Hygiene,' vol. 3, pp. 401, etc.
(5) v. Schrötter, 'Der Sauerstoff in der Prophylaxe, etc., der Luftdruckerkrankungen, etc.,' p. 71 etc.
(6) v. Schrötter, *op. cit.*, p. 49.
(7) Hill and Ham, "Phys. Soc. Proc.," 'Journ. Physiol.,' vol. 33.
(8) v. Schrötter, 'Zur Prophylaxe der sogenannten Taucherlahmung' (Congresso Internazionale per le Malattie del Lavoro).

The Inheritance of Flower Colour in Antirrhinum majus.

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(Communicated by W. Bateson, F.R.S. Received February 12,—Read February 21, 1907.)

[Plate 4.]

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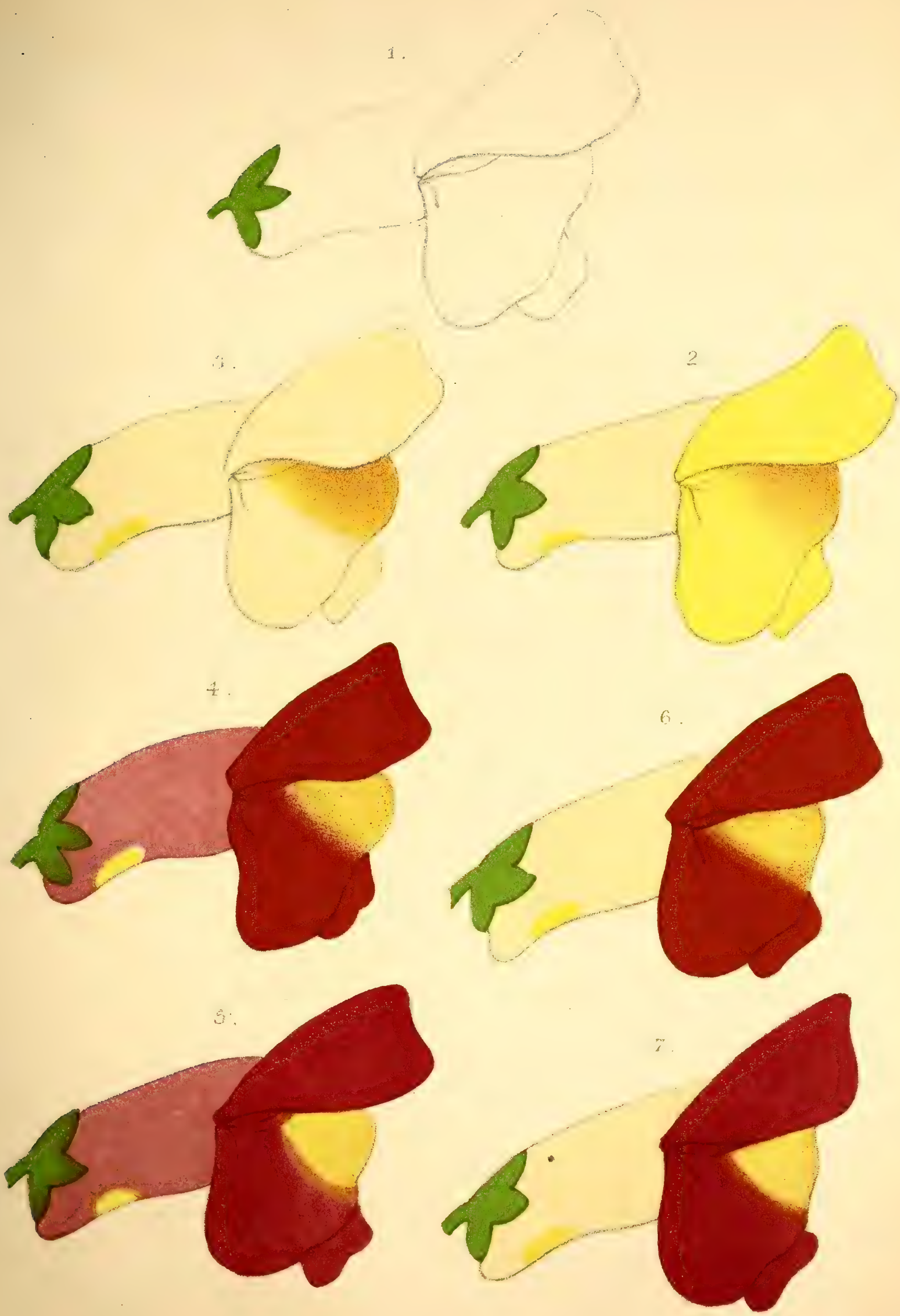
Introduction.

These experiments were begun in June, 1903. The plants used were raised from seed obtained from Messrs. Sutton. The course of inheritance in regard to flower colour was the main object of the enquiry.

Preliminary Statement of Results.

There are as regards colour five easily-distinguishable types of Antirrhinum flowers, namely:—

1. White—Lips and tube pure white (Pl. 4, fig. 1).
2. Yellow—Lips brimstone-yellow; tube ivory. The ivory tube is distinctly recognisable from the pure white tube of the albino (fig. 2).
3. Ivory—Lips and tube ivory. Ivory colour is due to a faint tingeing with yellow, and this type may be regarded as a very pale yellow, but the pigment is, perhaps, distinct from that which gives the yellow colour to Type 2 (fig. 3).
4. Crimson—Lips crimson; tube magenta (fig. 4).
5. Magenta—Lips and tube magenta (fig. 5).



Trap Lith^r. Leiden.

1. White. 2. Yellow. 3. Ivory. 4. Crimson. 5. Magenta.
6. Crimson delila. 7. Magenta delila.

The colours of all these types are sap-colours, not plastid-colours.

Forms of the crimson and magenta types occur in which the tube is ivory, while the lips are magenta or crimson respectively (figs. 7 and 6). The term "delila" was used by Professor De Vries* for such forms and has been adopted in this paper.

Yellow and magenta colours are produced by the corresponding sap-colours in the cells. Crimson colour in the lips is due to the simultaneous presence of magenta and yellow saps in the cells. That such is the case is easily seen in a microscopical examination of the tissue of the lips of a crimson flower. Groups of cells containing pure yellow or pure magenta saps are found among crimson cells in which the colour is undoubtedly due to a mixture.

With the exception of the albino type, the flowers of all types used have, irrespective of the colour of lips and tube, a constant orange-yellow palate; smaller patches similar in colour generally appear at the base of the tube on either side. This summer Mr. Bateson has shown me white types, some of which had a little orange-yellow colour on the palate, while others had in addition the yellow patches on the tube. Matings will be made with these types, but all whites employed and described in the experimental work have been entirely without colour.

The inheritance of the corolla colours can be represented by the following factors, which have been found to follow the Mendelian inheritance:—

Y.—A factor representing yellow† colour in the lips associated with ivory tube-colour.
I.—A factor representing ivory colour in the lips.
L.— „ magenta „ „
T.— „ „ „ in the tube.

Small letters, y, i, etc., denote the absence of these factors.

From experimental evidence it appears that:—

1. All zygotes, from which Y is absent, are white, though they may contain any of the factors I, L, and T.
2. The factor T is not manifested unless L is also present in the zygote; that is, no magenta colour appears in the tube unless magenta colour be also present in the lips.
3. All zygotes containing Y are coloured. The actual colour may be modified and determined by the presence of one or more of the remaining factors. A zygote containing Y only or Y and T is yellow.

---

* 'Mutationstheorie,' Lief. IV, pp. 194—206.

† This, of course, does not refer to palate colour, which may be regarded as a separate character.

4. Ivory is "dominant"[*] over yellow; a zygote containing Y and I or Y, I, and T, is ivory.
5. Since magenta superposed upon yellow gives crimson, a zygote containing Y, L, and T is crimson, Y and L only, crimson delila.
6. Magenta superposed upon ivory gives, since the latter is very pale, magenta. A zygote containing Y, I, L, and T is magenta, Y, I, and L only, magenta delila.

The five types described are readily distinguishable and quite discontinuous. Throughout the experimental work, all individuals met with have been at once assigned, without any difficulty, to one of the above classes. Magenta colour, however, appears in at least three distinct shades or degrees of concentration, which are probably determined by definite factors, though evidence on this point is not yet complete. Upon a yellow ground, these shades of magenta give the corresponding shades of crimson. In many cases deep magentas have been bred from the lighter magentas, so we must conclude that lighter forms are dominant to darker in the same sense that the pale form, ivory, is dominant to yellow. Similar phenomena have been found to occur in Sweet Peas and Stocks.[†]

The concentration of sap-colour in the tube and lips is generally approximately the same; the lips are somewhat darker in appearance, but this difference depends probably on texture and not on difference in depth of pigmentation. The tube may also be darker in shade than the lips, and this is the case in one or two types not yet investigated.

The magenta sap-colour sometimes appears in irregular fleckings or stripings, thus giving striped forms. If the striping occurs in the magenta type, the ivory ground-colour shows where the magenta sap fails and the flower may be described as ivory flecked or striped with magenta. When the magenta sap fails in the crimson type, the *yellow* ground-colour appears and the flower would be described as yellow flecked or striped with crimson. No flecking or striping of ivory on yellow has ever been observed.

In several cases, where striped and non-striped forms have been crossed, striping has disappeared in F_1, and has not appeared again either in F_2 or F_3. In another case of striped × non-striped (a paler magenta), the deeper striping persisted in F_1 on the paler magenta ground; a striped F_1 has been given also in the mating of two striped forms. In the two latter cases the results are not yet complete.

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[*] "Dominant" is here used to denote that the lighter colours overlie or suppress the darker, as ivory, for example, conceals yellow; and not in its strict sense of expressing the relationship between a pair of allelomorphic characters: ivory and yellow are not, of course, allelomorphic to each other.

[†] 'Third Report to the Evolution Committee,' W. Bateson, E. R. Saunders, and R. C. Punnett, p. 4.

The original wild Antirrhinum appears to be of the magenta type. "Reversion" to this old form, which so often happens when crimson, ivory, or yellow types are crossed with white, is explained by the introduction into the zygote of the factors I, L (and T) by the white parent. The introduction of the factor I alone will bring about "reversion" when L is already present in the zygote.

The experimental evidence is not yet sufficient to determine whether the phenomena in Antirrhinum are fundamentally similar to those observed for Sweet Peas and Stocks. In the two latter cases,* production of colour depends on the simultaneous presence in the zygote of two factors C and R; the absence of both or either of these factors from the zygote renders it white. The colour formed by the meeting of the two factors is red.

To treat the case of Antirrhinum on parallel lines, we might suppose the production of yellow, the base-colour, so to speak, to be due to the presence in the zygote, not of one factor only, but of two factors. Any zygote containing both would be yellow; one only or neither would be white. If such is the case, whites should be found, which, when crossed together, give yellow. Whether this is the case or not cannot be decided yet, since the results of crosses between whites are not known.

All phenomena so far observed in Antirrhinum could be equally well represented on the two-factor theory, provided we assume that the whites, originally mated with coloured types, contained one of the factors which constitute colour; this is quite possible, since so few white *individuals* (only three) were employed in the matings.

The question, then, as to whether colour in Antirrhinum depends on the meeting of two complementary factors or not, must remain open for the present. If two factors be necessary, albinos should be found, which, when mated, give colour (here yellow), as in the cases of Sweet Peas and Stocks; if, on the other hand, only one factor is necessary, then no two albinos when mated will ever produce colour, and the case will be analogous rather to the phenomena observed in animals.

In the Sweet Peas and Stocks, moreover, the colour of the zygote may be further determined by the presence of a third blue factor B, which changes the red colour to purple; but B has no effect unless C and R are also present, and thus may be carried by an albino without producing a sensible effect.

Quite comparable to this third factor in Peas and Stocks are the factors I,

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* 'Third Report.

L, and T in Antirrhinum, none of which produce a sensible effect unless yellow is also present. Any of these may be carried by an albino.

The factor I modifies yellow, giving ivory. The factor L, superposed upon ivory, gives magenta ; with yellow it blends, giving crimson ; finally, the third factor T is not manifested unless L in addition is present.

Details of Various Matings.

WHITE × OTHER TYPES.

White × Yellow Crimson-striped:—

The type yellow crimson-striped is, as explained above, really crimson, in which the magenta sap occurs in irregular stripings and fleckings, so that the lips appear yellow striped with crimson and the tube ivory striped with magenta; a corresponding delila form exists, having an ivory tube.

White has always been found to breed true, but the selfings of the other parent unfortunately failed, so that its constitution can only be deduced from the results of crossing.

F_1 consisted of magentas and magenta delilas (Table I, p. 303).

F_2—The cross-breds appeared to be of four kinds, giving respectively, on selfing:—

	Case 1. Magenta × self.	Case 2. Magenta × self.	Case 3. Magenta delila × self.	Case 4. Magenta delila × self.
Magenta	107	229	—	—
„ deliia ...	42	70	59	47
Crimson............	42	74	—	—
„ delila ...	12	19	18	5
Ivory	67	—	19	—
Yellow	24	—	7	—
White	80	97	34	19
Totals	374	489	137	71

If we assume the original parents to be:—

yy II LL tt .... White
YY ii Ll Tt..................... Yellow crimson-striped,

then the cross-breds would be of four kinds:—

Yy Ii Ll Tt..................... Magenta (Case 1)
Yy Ii LL Tt .... „ (Case 2)
Yy Ii Ll tt .... „ delila (Case 3)
Yy Ii LL tt .... „ „ (Case 4),

whole forms and delilas occurring in equal proportions. "Reversion" is here due to the introduction of I by the white parent.

From Case 1, on selfing, we should expect to obtain theoretically as the result of the combination of four pairs of characters:—

		Ratios.	Numbers calculated.	Numbers observed.
Y I L T ...	Magenta	81 or	118·33	107
Y I L	„ delila ...	27	39·44	42
Y I T	Ivory	36	52·59	67
Y L T	Crimson............	27	39·44	42
Y I	Ivory	—	—	—
Y T	Yellow	12	17·53	24
Y L	Crimson delila ...	9	13·15	12
Y	Yellow	—	—	—
—............	White	64	93·50	80
	Totals	256	373·98	374

From Case 2 we should obtain theoretically:—

		Ratios.	Numbers calculated.	Numbers observed.
Y I T L ...	Magenta	27 or	206·30	229
Y I L	„ delila...	9	68·76	70
Y T L	Crimson............	9	68·76	74
Y L	„ delila...	3	22·92	19
—............	White	16	122·25	97
	Totals	64	488·99	489

From Case 3:—

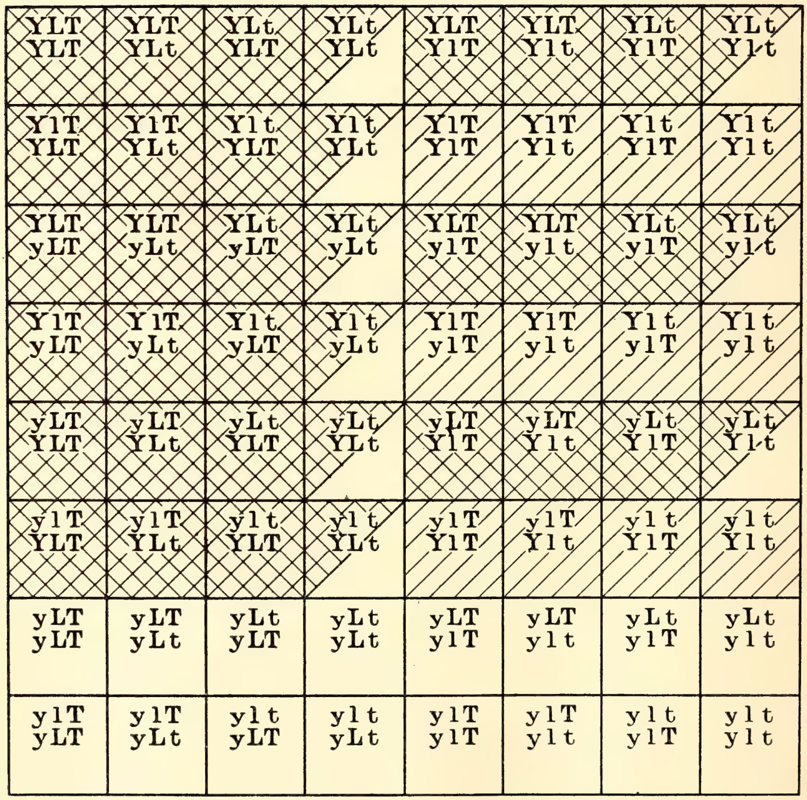
		Ratios.	Numbers calculated.	Numbers observed.
Y I L	Magenta delila ...	27 or	57·80	59
Y I	Ivory	9	19·26	19
Y L	Crimson delila ...	9	19·26	18
Y	Yellow	3	6·42	7
—............	White	16	34·25	34
	Totals	64	136·99	137

And from Case 4:—

		Ratios.	Numbers calculated.	Numbers observed.
Y I L	Magenta delila...	9 or	39·94	47
Y L.........	Crimson „ ...	3	13·31	5
—...........	White	4	17·75	19
	Totals	16	71·00	71

The close agreement between the theoretical and experimental numbers justifies the assumptions made in regard to the constitution of the original parents.

Diagram I represents the composition of F_2 in Case 1 when we consider



YLT YLT	YLT YLt	YLt YLT	YLt YLt	YLT YlT	YLT Ylt	YLt YlT	YLt Ylt
YlT YLT	YlT YLt	Ylt YLT	Ylt YLt	YlT YlT	YlT Ylt	Ylt YlT	Ylt Ylt
YLT yLT	YLT yLt	YLt yLT	YLt yLt	YLT ylT	YLT ylt	YLt ylT	YLt ylt
YlT yLT	YlT yLt	Ylt yLT	Ylt yLt	YlT ylT	YlT ylt	Ylt ylT	Ylt ylt
yLT YLT	yLT YLt	yLt YLT	yLt YLt	yLT YlT	yLT Ylt	yLt YlT	yLt Ylt
ylT YLT	ylT YLt	ylt YLT	ylt YLt	ylT YlT	ylT Ylt	ylt YlT	ylt Ylt
yLT yLT	yLT yLt	yLt yLT	yLt yLt	yLT ylT	yLT ylt	yLt ylT	yLt ylt
ylT yLT	ylT yLt	ylt yLT	ylt yLt	ylT ylT	ylT ylt	ylt ylT	ylt ylt

Diagram I.—Scheme of Distribution of three of the Colour-factors in F_2 from white × yellow crimson-striped.

Cross-hatched squares = crimson-flowered plants.
Single-hatched „ = yellow „ „
Half plain „ = crimson delila flowered plants.
Plain „ = white-flowered plants.

three factors only; the introduction of a fourth factor would give magenta and ivory individuals.

A similar diagram constructed for four factors shows us that, as regards the composition of the F_3 they give on selfing, there are 16 kinds of magentas in F_2. Among 13 magenta plants taken at random in F_2 and selfed, 8 out of the 16 kinds were found. (For Case 1, Table II gives F_3 resulting from all the F_2 individuals selfed.) The remaining 8 would doubtless occur among a larger selection of F_2 individuals.

Of magenta delilas, there are theoretically 8 kinds; 6 plants taken at random gave 2.

Of crimsons, theoretically 8; 5 plants gave 3.
Of crimson delilas, 4; 3 plants gave 2.
Of ivories, 4; 6 plants gave 3.
And of yellows, 2; 3 plants gave both.

A corresponding diagram can easily be constructed for Case 2.

From such a diagram one learns that the F_2 magentas are theoretically of 8 kinds; 11 plants taken at random gave 7 out of the 8. (Table III gives F_3 for Case 2.)

Of magenta delilas, 4; 4 plants gave 4.
Of crimsons, 4; 6 plants gave 4.
Of crimson delilas, 2; only 1 plant was taken.
Two white plants gave whites only.

Similarly, from a diagram constructed for Case 3, we find there are 8 possible magenta delilas; 5 plants gave 5. (Table IV gives F_3 for Case 3.)

Of crimson delilas, 4; 3 plants gave 2.
Of ivories, 4; 1 plant only was taken.
Of yellows, 2; 1 plant only was taken.

Finally, from a diagram for Case 4, we find there are 4 possible magenta delilas; 6 plants gave 2. (Table V gives F_3 for Case 4.) One white plant gave whites only. No other F_2 plants were taken.

It is clear also from Diagram I that there are 8 possible albinos. Various yellows this year have been crossed with a number of F_2 whites as a means of testing for the characters which may be borne by an albino.

White × *Crimson Delila*:—

Both these types were found to be breeding true.

F_1 consisted of magenta delilas only. (Table I.)

F_2—Two of the cross-breds were selfed, the combined result giving:—

	Number.
Magenta delila	89
Crimson „	34
White	41
Total	164

If we assume the original parents to be:—

yy II LL tt .... White
YY ii LL tt .... Crimson delila,

the cross-breds would be of one kind only:—

Yy Ii LL tt .... Magenta delila

"Reversion" is again due to the introduction of I by the white plant.

From selfing the cross-bred we should obtain theoretically:—

		Ratios.	Numbers calculated.	Numbers observed.
Y I L	Magenta delila ...	9 or	92·25	89
Y I L	Crimson „ ...	3	30·75	34
—............	White	4	41·00	41
	Totals	16	164·00	164

There is a close agreement between the theoretical and experimental numbers.

White × *Crimson*:—

The crimson parent was hybrid as regards the characters L and T. F_1, as would be expected, consisted of magentas and magenta delilas. (Table I.) Except as regards striping, this is a case similar to white × yellow crimson-striped, and the same series of types were obtained in F_2 as in the latter case. As yet, sufficient numbers have not been grown to make the details complete.

White × *Yellow*:—

Both parents were breeding true.

F_1 consisted of magenta delilas only. (Table I.)

If we represent the parents by:—

yy II LL tt .... White
YY ii ll tt........................... Yellow,

then the cross-breds would be of one kind only :—

Yy Ii Ll tt .... Magenta delila

F_2 has not been obtained yet.

CRIMSON DELILA × OTHER TYPES.

Crimson Delila × Yellow Crimson-striped :—

The crimson delila parent was breeding true. The other parent was the same individual used in the cross white × yellow crimson-striped.

F_1 consisted of crimsons and crimson delilas. (Table I.)

F_2—The cross-breds appeared to be of four kinds, giving respectively, on selfing :—

	Case 1. Crimson × self.	Case 2. Crimson × self.	Case 3. Crimson delila × self.	Case 4. Crimson delila × self.
Crimson...........	56	35	—	—
„ delila ...	24	17	20	23
Yellow	32	—	8	—
Totals	112	52	28	23

If we assume the original parents to be :—

YY ii LL tt .... Crimson delila
YY ii Ll Tt .... Crimson,

then the cross-breds would be represented by :—

YY ii Ll Tt........................ Crimson (Case 1)
YY ii LL Tt .... „ (Case 2)
YY ii Ll tt .... „ delila (Case 3)
YY ii LL tt...................... „ „ (Case 4),

whole forms and delilas occurring in equal proportions.

From Case 1, on selfing, we should obtain theoretically :—

		Ratios.	Numbers calculated.	Numbers observed.
L T Y......	Crimson...........	9 or	63·00	56
L Y..........	„ delila ...	3	21·00	24
T Y..........	Yellow	4	28·00	32
Y............	„	—	—	—
	Totals	16	112·00	112

From Case 2:—

		Ratios.		Numbers calculated.	Numbers observed.
T Y L......	Crimson............	3	or	39 ·00	35
Y L.........	„ delila ...	1		13 ·00	17
	Totals	4		52 ·00	52

From Case 3:—

		Ratios.		Numbers calculated.	Numbers observed.
L Y.........	Crimson delila ..	3	or	21 ·00	20
Y	Yellow	1		7 ·00	8
	Totals	4		28 ·00	28

And from Case 4 we should get crimson delilas only, which agrees with the experimental result.

In Case 1 there are theoretically 4 kinds of F_2 crimsons; 10 plants taken at random gave 3 kinds. (Table VI gives F_3 for Case 1.)

Of crimson delilas there are two kinds; both were given by 4 plants.

Yellows are of 1 kind only; 8 yellows gave yellows only.

Crimson Delila × *Crimson*:—

The crimson delila parent was breeding true. The crimson parent was shown to be hybrid as regards T, giving, on selfing, 3 crimsons to 1 crimson delila.

F_1 consisted of crimsons and crimson delilas. (Table I.)

F_2—Three crimson delila cross-breds gave crimson delilas only. F_2 has not yet been obtained from the crimsons.

If we assume the parents to be:—

YY ii LL Tt .... Crimson
YY ii LL tt .... „ delila,

the cross-breds would be:—

YY ii LL Tt .... Crimson
YY ii LL tt .... „ delila,

whole forms and delilas in equal proportions, and the latter would give crimson delilas only on selfing, which agrees with the experimental results.

Crimson Delila × *Yellow* :—

Both parents were breeding true.

F_1 gives the expected result, namely, all crimson delilas. (Table I.)

F_2 has not yet been obtained.

CRIMSON × OTHER TYPES.

Crimson × *Yellow Crimson-striped* :—

The crimson parent was breeding true. The other parent was the plant used in previous crosses.

F_1 consisted of crimsons only. (Table I.)

F_2—Only one cross-bred gave seed and the offspring were crimsons and yellows. The numbers are too small to be of value.

If we assume the original parents to be :—

YY ii LL TT .... Crimson
YY ii Ll Tt .... Yellow crimson-striped,

the cross-breds would be :—

YY ii Ll TT .... Crimson
YY ii Ll Tt..................... „
YY ii LL TT .... „
YY ii Ll Tt..................... „

All therefore are crimsons, and of these, one, on selfing, namely, YY ii Ll TT, would give crimsons and yellows.

Crimson × *Yellow* :—

F_1 gave the expected result, namely, all crimsons. (Table I.)

F_2 has not yet been obtained.

ROSE × OTHER TYPES.

The type rose has ivory lips tinged with magenta and a pale magenta tube. The individuals used in crossing were found to breed true. Crosses were made between this and most of the other types, but sufficient numbers have not been obtained in F_2 to give a reliable indication of the results. A type, where " rose," *i.e.*, a tingeing with magenta, is superposed upon a yellow ground (the case where the factor I is missing), appeared in F_2, together with delila forms of both types; this would lead to the supposition that " rose " may be represented by a Mendelian factor.

An interesting point has arisen in connection with the cross rose × a deep crimson delila. The cross-bred is a deep magenta, in which the tube takes the same shade as the lips. This fact suggests, as pointed out elsewhere (p. 301), that there may be a coupling between the shade of tube- and lip-colour; magenta colour introduced into the tube takes the same concentration as that in the lips.

Experiments of De Vries on Antirrhinum.

In the 'Mutationstheorie,' Lief. IV, pp. 194—206, Professor De Vries gives an account of experiments in crossing certain varieties of *Antirrhinum majus.* It is stated that yellow colour was not taken into consideration and experiments were confined to the white and red varieties. The following is a description of the types mentioned:—

Roth	Tube and lips red, the lips deeper
Fleischfarbig ...	Tube and lips pale red
Delila............	Tube pale or white, lips fairly deep red
Weiss............	White, often with a distinct very pale red tinge

Professor De Vries regards red as being made up of *fleischfarbig* F and *delila* D, two dominant characters, and white as producing two characters, w and w′, recessive to F and D respectively.

A red Antirrhinum was crossed with white, giving an F_1 like the red parent. From the F_1 cross-breds on selfing, four types were obtained:—the original red, delila, flesh-colour, and the original white, roughly in the proportions 9 : 3 : 3 : 1. This result would be represented:—

Red × white
FD × ww′
↓
FDww′ Red cross-bred

The cross-bred × self would give:—

		Ratios.		Per cent.
FD	Red	9	or	56
F	Flesh-colour	3		19
D	Delila	3		19
ww′	White	1		6
	Total	16		100

The numbers resulting from three experiments were:—

Red	51	58	58
Flesh-colour ...	16	17	11
Delila	31	20	21
White	2	4	10
Totals	100	99	100

The reds in F_2 are of four kinds and would on self-fertilisation give in F_3 the following results :—

1 FD → FD red
2 FDw′ → FD (red) + Fw′ (flesh-colour)
2 FDw → FD (red) + Dw (delila)
4 FDww′ → the whole series again

The delilas are of two kinds :—

1 Dw → Dw (delila)
2 Dww′ → Dw (delila) + ww′ (white)

The flesh-colours also :—

1 Fw′ → Fw′ (flesh-colour)
2 Fww′ → Fw (flesh-colour) + ww′ (white)

Professor De Vries showed that a delila and a flesh-colour in F_2 gave, on selfing, an F_3 consisting of delilas only and flesh-colours only respectively. Other F_2 delilas gave delilas and whites and other F_2 flesh-colours gave flesh-colours and whites. Further, among the F_2 reds, some gave reds and flesh-colours in the proportion 3 : 1, while others gave reds and delilas in the same proportion.

Professor De Vries has kindly informed me that his types correspond to the following types mentioned in this paper, namely :—

Roth	to	Magenta
Delila	„	„ delila
Fleischfarbig	„	Rose
Weiss	„	Ivory
„ (tinged)	„	Rose delila

Rose × a delila form has been found to give a deep magenta whole form (p. 299) and the result is consistent with the supposition that there is an interdependency between the shade of colour in lips and tube ; the magenta tube-colour, introduced by rose, takes, in the cross-bred, the same shade as the lip-colour. This result corroborates Professor De Vries' suggestion that *roth* consists of *fleischfarbig* and *delila.**

Translated into the types used in this paper, the simplest expression for

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* [In 'Camb. Phil. Soc. Proc.,' vol. 12, 1902, p. 50, I suggested an interpretation of De Vries' results with Antirrhinum, which then seemed to me preferable to that put forward by him. Further evidence as to this and other cases of the resolution of compound characters has shown that my suggestion was incorrect (see also 'Rep. Evol. Com.,' III, p. 12).—W. BATESON.]

Professor De Vries' parent plants would be (Rl = rose lips, Rt = rose tube):—

YY II Rl Rl LL Rt Rt...... Magenta (Rl Rt L = magenta)
YY II rl rl ll rt rt .... Ivory

and the cross-breds:—

YY II Rl rl Ll Rt rt......... Magenta

would give, on selfing:—

		Ratios.	Numbers calculated.	Numbers observed.
			per cent.	per cent.
Rl L Rt ...	Magenta	36 or	56	56
Rl L	„ delila	12	19	24
Rl Rt	Rose	9	14	15
L Rt	Magenta	—	—	—
Rl	Rose delila	3 } 7	11	5
L	Magenta delila......	—		
Rt	Ivory	4		
—............	„	—	—	—

The ivories and rose delilas are classed together as De Vries' whites and tinged whites. The numbers observed are the mean of those given for the three experiments; moreover, the various kinds of *roth* and *fleischfarbig* would be found among the above F_2 forms.

In conclusion, I wish most sincerely to thank Miss Saunders and Mr. Bateson for the advice and help they have kindly given me, both as regards many practical details and in the writing of this paper.

I am also indebted to Mr. Lynch, of the Botanic Gardens, Cambridge, for allowing me space under cover every year for the germination of my seedlings.

Table I.—F_1 from Matings between various Types.

Matings.	Colours in F_1.				
	Magenta.	Magenta delila.	Crimson.	Crimson delila.	Rose.
White × yellow crimson-striped ...	24	19*			
„ × crimson delila	...	33			
„ × crimson	4	4			
„ × yellow	...	21			
Crimson delila × yellow crimson-striped	...	...	36	35	
„ × crimson	...	...	8	10	
„ × yellow	...	...	...	22	
Crimson × yellow crimson-striped...	...	...	36		
„ × yellow	...	...	23		
Rose × white	184				
„ × crimson	25				
„ × crimson delila	30				
„ × yellow crimson-striped	19				
„ × yellow	...	...	...	...	20
„ × ivory	...	...	...	...	20 (paler)

* The small total here may be due to the fact that a large number of F_1 individuals were destroyed by frost.

Table II.—F_3 from Case 1 in the Mating White × Yellow Crimson-striped.

Number of F_2 individuals.	Colour of F_2 individuals.	Colours in F_3.						
		Magenta.	Magenta delila.	Crimson.	Crimson delila.	Ivory.	Yellow.	White.
3	Magenta	+	+	+	+	+	+	+
1	„	+	+	+	+	+	+	
3	„	+	...	+	...	+	+	+
1	„	+	+	...	...	+		
2	„	+	...	+	...	+	+	
1	„	+	+	...	...	...	...	+
1	„	+	...	...	...	+		
1	„	+	+	+	+	...	...	+
5	Magenta delila	...	+	...	+	+	+	+
1	„ „	...	+	...	...	+	...	+
2	Crimson	...	...	+	+	...	+	+
1	„	...	...	+	...	...	+	+
2	„	...	...	+	...	...	+	
2	Crimson delila	...	...	...	+	...	+	
1	„ „	...	...	...	+	...	...	+
2	Ivory	...	...	...	...	+	+	
2	„	...	...	...	...	+	...	+
2	„	...	...	...	...	+		
2	Yellow	...	...	...	...	...	+	+
1	„	...	...	...	...	...	+	

Table III.—F_3 from Case 2 in the Mating White × Yellow Crimson-striped.

Number of F_2 individuals.	Colour of F_2 individuals.	Colours in F_3.				
		Magenta.	Magenta delila.	Crimson.	Crimson delila.	White.
2	Magenta	+	+	+	+	+
2	„	+	+	+	+	
3	„	+	+	...	...	+
1	„	+	+			
1	„	+	...	+	...	+
1	„	+	...	+		
1	„	+				
1	Magenta delila	...	+	...	+	+
1	„ „	...	+	...	+	
1	„ „	...	+	...	...	+
1	„ „	...	+			
1	Crimson............	...	...	+	+	+
1	„	...	...	+	...	+
2	„	...	...	+	+	
2	„	...	...	+		
1	Crimson delila	...	...	...	+	+
2	White	...	...	...	...	+

Table IV.—F_3 from Case 3 in the Mating White × Yellow Crimson-striped.

Number of F_2 individuals.	Colour of F_2 individuals.	Colours in F_3.				
		Magenta delila.	Crimson delila.	Ivory.	Yellow.	White.
1	Magenta delila	+	+	+	+	+
1	„	+	+	+	+	
1	„	+	+	...	...	+
1	„	+	...	...	...	+
1	„	+	...	+		
2	Crimson delila	...	+	...	+	
1	„	...	+	...	...	+
1	Ivory	...	...	+	+	+
1	Yellow	...	...	...	+	+

Table V.—F_3 from Case 4 in the Mating White × Yellow Crimson-striped.

Number of F_2 individuals.	Colour of F_2 individuals.	Colours in F_3.		
		Magenta delila.	Crimson delila.	White.
2	Magenta delila	+	+	
4	„	+	...	+
1	White...........	...	...	+

Table VI.—F_3 from Case 1 in the Mating Crimson Delila × Yellow Crimson-striped.

Number of F_2 individuals.	Colour of F_2 individuals.	Colours in F_3.		
		Crimson.	Crimson delila.	Yellow.
5	Crimson	+	+	+
3	"	+	...	+
2	"	+		
2	" delila	...	+	+
2	" "	...	+	
8	Yellow	...	...	+

Longitudinal Symmetry in Phanerogamia.

By Percy Groom, M.A., D.Sc., F.L.S.

(Communicated by Dr. D. H. Scott, F.R.S. Received January 24,—Read February 21, 1907.)

(Abstract.)

The first object of this communication is to put forward a simple *graphic method* of recording measurements concerning the longitudinal symmetry of plants, and to test its efficiency as a means of solving morphological problems. The method is as follows:—Measurements of the successive internodes of a stem are made, and are recorded on squared paper as successive ordinates; the resultant curve is termed the *internode curve.* The longitudinal distances apart of other members are dealt with in the same manner, and ranged into other curves.

The second object of the paper is to describe the general and particular results obtained by use of the method.

In a *typical herb,* the internode curve of the main axis is a regular and characteristic ascending-descending one (see Curve 1 in fig. 1), while those of

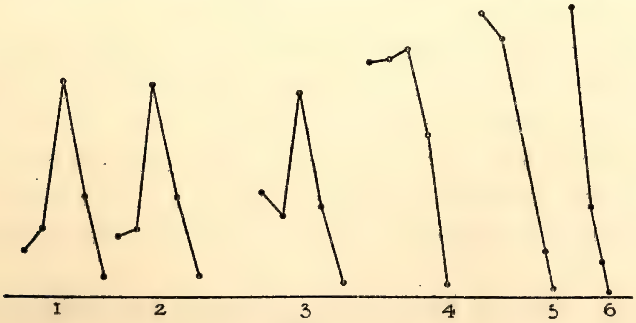

Fig. 1.

the successive branches, commencing at the base of the plants and ascending the main stem, gradually change from the ascending-descending curve to a purely descending one (see Curves 2—6 in fig. 1), or tend to do so. It is this gradual change in the internode curve of the successive branches that explains the two maxima in the internode lengths (and possibly in the rate of growth) met with in branches.

By reference to plants of different habit, and growing under different climates or in different habitats (including water), it is proved that these internode curves are characteristic of the species, and inherent though liable to disturbance by external conditions.

Alternate-leaved Chenopodiaceæ, both in the main axis and in the branches, display a remarkable periodic zig-zag in the internode curve (see the curve printed plain in fig. 2). By connecting the alternate ordinates, this can be analysed into two consistent and often regular sub-curves (see the dotted curves in fig. 2); one of these sub-curves is the "internode sub-curve," and the other the "displacement sub-curve." By comparison of the curves of the main axis and branches of Chenopodiaceæ and typical herbs, also between those of alternate-leaved and opposite-leaved Chenopodiaceæ, as well as by other means, it is shown that the displacement sub-curve represents the distances up which one leaf at each successive node has been relatively displaced from a primitively opposite arrangement. Both sub-curves of the main axis assume the ascending-descending form, but in the successively higher branches the internode sub-curve changes from the ascending-descending form to a purely descending one, while the displacement sub-curve tends to do likewise, or does so, at a later stage. This inherent peculiarity of the chenopodiaceous internodes sheds light upon the affinity of the family to the Caryophyllaceæ, whose opposite leaves do not develop simultaneously; and there are indications that at least several, if not all, of the families comprising the Centrospermæ are opposite-leaved in reality, or, like the Chenopodiaceæ, in design.

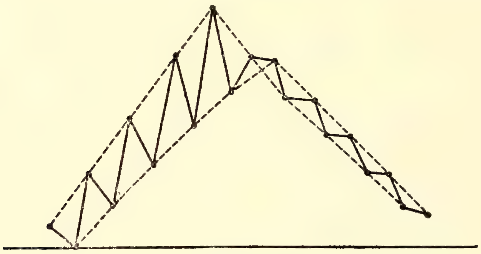
Fig. 2.

To test this theory of displacement, reference was made to the admitted case of displacements of branches above their leaf-axils in *Boraginaceæ.* In this connection, the curve was constructed by recording as successive ordinates the heights of the successive branches above their subtending leaves. The resultant displacement curve of the branches is of startling regularity, and conforms in type with the displacement sub-curve of the leaves on the chenopodiaceous main stem.

One significant point is that these old-established fixed (hereditary) displacements in Chenopodiaceæ and Boraginaceæ follow the same *rule* in respect to their distribution and relative dimensions as that formulated by De Vries *in respect to anomalies and monstrosities.*

The Boraginaceæ are shown to be probably opposite-leaved in design, though alternate-leaved by displacement, and are thus brought into closer connection with the Labiatæ.

In the advanced case of *Solanum Dulcamara,* the graphic method showed for the first time that the familiar displacements of leaves high up the axis are anticipated lower down by similar ones.

Investigations of *slighter or more fluctuating leaf-displacements* in *Lysimachia vulgaris, Veronica virginiana,* and *Œnothera,* showed that where the phyllotaxis is sometimes cyclic, and at other times acyclic, on the same axis, individual, or species, the internode curve becomes irregular when any of the leaves are alternate or sub-opposite. This irregular curve could be analysed into consistent sub-curves when the displacements were slight. But in some instances of more considerable displacements I failed to accomplish this analysis, partly because the number of leaves originally associated with a node is apt to vary at different points of the same axis. The irregularity of the curve is due to two causes: (1) the method of measurement rigidly adopted, namely, from one leaf to the next one, may obviously lead to a merely seeming diminution in length of an internode; (2) but, beyond this, the displacement of a leaf causes a true (relative) abbreviation of the internode up which it takes place. The irregularity of the internode curve in these cases, and in others investigated, is of import in indicating that the construction of an internode curve of merely one specimen (living or fossil) may suffice to show that the phyllotaxis in the family varies from acyclic to cyclic, just as similar treatment of one axis of a herb (living or fossil) may show at once whether the axis be a main one or a branch and, if the latter, it may give some information as to its insertion on the mother axis.

Incidentally, observations were made on the *double-leaves* of *Lysimachia vulgaris* and *Rhinanthus,* and on the increase and decrease in the number of leaves at a node in these plants. The morphological significance of the observed facts is discussed. Of particular interest and novelty was one case in *Rhinanthus,* in which the opposite phyllotaxis changed to alternate by a process which some botanists would describe as one of the concrescence of the two opposite leaves.

The observations just mentioned, as well as some regarding displacements, more or less clearly indicated than when phyllotaxis is cyclic in design, alternate internodes, nodes, and associated displacements are more closely correlated than are successive ones. This may imply that morphogenous impulses travel along orthostichies, but it at least sheds light upon a number of varied morphological problems.

The theory of the *construction of the phanerogamous stem* is discussed, and stress is laid upon the fact that the longitudinal displacement of leaves demonstrates that internodes may be of different phylogenetic age and lineage, and are, therefore, not serially homologous segments. The interpretation of these displacements adopted in this paper would also imply that the stem is partly constituted of tissue originally appertaining to leaves, so that " stem " and " leaf " are not sharply distinguished members.

The graphic method was also tested by reference to *other morphological problems.* It does not afford the means of distinguishing between a sympode and a monopode in the cases of the sympodes of *Petunia* and *Myosotis,* though the curves harmonise with the accepted view. But more decisive results were obtained with the tendrilled axis of *Ampelopsis hederacea;* here the complex, periodic, yet apparently irregular, internode curve could be analysed only into three consistent sub-curves (by joining every third ordinate); and this exactly corresponds to the interpretation of structure adopted by Eichler and others, while it directly conflicts with rival theories.

The Estimation of Chloroform in the Blood of Anæsthetised Animals.

By G. A. Buckmaster, Assistant Professor of Physiology, University College, University of London, and J. A. Gardner, Lecturer on Physiological Chemistry, University of London.

(Communicated by Dr. A. D. Waller, F.R.S. Received February 1,—Read February 21, 1907.)

(From the Physiological Laboratory of the University of London.)

In a former paper* we expressed doubt whether the methods employed by Dr. Maurice Nicloux and other French observers for accurately determining the percentage of chloroform in the blood of anæsthetised animals were capable of yielding such satisfactory results as those obtained by Nicloux when known quantities of chloroform were added to blood *in vitro;* on the ground that the mixing of blood and chloroform *in vitro* is an essentially different process from that which takes place during the physiological process of inhalation, since it has been shown that, during anæsthesia, chloroform associates itself particularly with the red corpuscles.

In order to test the validity of our criticism, and to enable the results obtained by the French observers to be correlated with our own, and also to gain an idea as to how far the method employed by Nicloux is capable of giving reliable results in the case of tissues to which our method may not be readily applicable, we have made a series of parallel estimations by the two methods on the blood of anæsthetised animals.

In our experiments with the method of Nicloux, we followed closely the procedure which he has detailed†, and we take this opportunity to express our indebtedness to Dr. Nicloux for his kindness in supplying us with the distillation-apparatus which he uses, and in giving us a demonstration of his method. As Nicloux points out,‡ the chief difficulty in his method consists in determining exactly the end-point of the reaction which occurs in titrating with silver nitrate, using potassium chromate as an indicator. While taking every care to hit the end-point as closely as possible, we uniformly slightly over-stepped rather than under-stepped the mark. In carrying out the method, we found it necessary to use caustic soda made from sodium instead of caustic potash as recommended by Nicloux, as we were unable in this country to obtain potash absolutely free from chloride.

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* 'Roy. Soc. Proc.,' B, vol. 78, p. 418, 1906.

† "Dosage de petites quantités de chloroforme," 'Extraits du Bulletin de la Société Chimique de Paris,' 3rd series, vol. 33, p. 321, 1906.

‡ *Ibid.*

I. *Analyses of Chloroform by the Carius and by the Nicloux Method* (*Dumas' Reaction*).

The chloroform used in these experiments was kindly distilled for us by Dr. J. Wade of Guy's Hospital. It had a boiling-point of 61·14°—61·15° C. at 760 mm. and a specific gravity 1·5088 15°/15°. The results of the analyses are given in Table I and Table II.

Table I (Carius' determinations).

Amount of chloroform taken.	Percentage of chlorine.	Theoretical percentage of chlorine.	Percentage of chloroform found.
gramme. 0 ·1177	89 ·172	89 ·102	100 ·07
0 ·2721	89 ·139	89 ·102	100 ·03
0 ·2683	89 ·084	89 ·102	99 ·97

Table II (Nicloux' determinations).

	Amount of chloroform taken.	Percentage of chlorine found.	Theoretical percentage of chlorine.	Percentage of chloroform found.
I. Made with a solution of 0 ·92 per 1000 c.c. of alcohol	gramme. 0 ·0184	89 ·262	89 ·102	100 ·18
II. Made with a solution of 1 ·575 per 1000 c.c. of alcohol	0 ·0315	88 ·041	89 ·102	98 ·8
III. Made with a solution of 1 ·6764 per 1000 c.c. of alcohol	0 ·0335	87 ·935	89 ·102	98 ·69
IV. Made with a solution of 3 ·3556 per 1000 c.c. of alcohol	0 ·06785	86 ·66	89 ·102	97 ·26
V. Made with a solution of 9 ·3675 per 1000 c.c. of alcohol	0 ·018735	90 ·54	89 ·102	101 ·6
VI. Do. do.	0 ·04684	87 ·378	89 ·102	98 ·06

From the second table it will be seen that the average percentage of chlorine found by the Nicloux method from the sample of chloroform is 88·3, a figure which is 0·8 per cent. below the theoretical value. The figures are comparable with those given in the following table taken from Nicloux' paper.

	Poids de chloroforme.		Quantité de chloroforme retrouvé.
	Mis.	Trouvé.	
	millegrammes.	millegrammes.	par cent.
I. Faite avec une solution alcoolique de chloroforme à 10 grammes par litre	5	5·0	100·0
	10	9·8	98·0
	20	19·4	97·0
	50	48·6	97·0
	100	96·0	96·0
II. Faite avec une solution alcoolique de chloroforme à 2 grammes par litre	4	4·0	100·0
	10	9·9	99·0
	20	19·7	98·5
	40	39·8	98·5

II. *Determination of Chloroform in the Arterial Blood of Anæsthetised Animals by our Method and that of Nicloux (Dumas' Reaction).*

The experiments were conducted on cats. The animals were anæsthetised by ether, and the necessary operations for introducing cannulæ into the trachea and carotid artery were performed. The tracheal cannula was fitted to a Chauveau's valve and chloroform administered by a Woulff's bottle. Samples of blood were taken before the administration of chloroform, and at some selected period in the course of the anæsthesia a measured quantity of blood (20 to 30 c.c.) was withdrawn, and this was *at once* divided up into various proportions for analysis by the two methods. For the purpose of the Nicloux estimations some samples were allowed to clot before estimation,* some were treated with sodium oxalate to prevent clotting, others with hirudin, and others were defibrinated by shaking with mercury. In the case of blood withdrawn at the asphyxial point it is, in the case of cats, very difficult to weigh any sample before clotting takes place, since this occurs with great rapidity.

1. *Experiments in which the Blood was allowed to clot before treatment with acidified Alcohol.*

Varying weights of blood were taken on which to carry out duplicate experiments. During distillation, the clotted blood was found to become detached as a disc of leathery consistence, about the size and thickness of a four-shilling piece, which persisted to the end of the process. The formation of this leather-like mass was only partially prevented by breaking up the clot with a rod before distillation. The results of these experiments are given in the following table.

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* Dr. Nicloux has kindly informed us, by letter, of a detail that we did not gather from his paper, viz., that in his observations the blood is withdrawn with a syringe and immediately mixed with alcohol so that it does not clot *en masse*.

Table III.

Number of experiment.	Weight of blood taken.	State of anæsthesia.	Chloroform by Carius.	Chloroform by Nicloux.
	grammes.			
1	5 ·954	Asphyxia	0 ·053	
	7 ·925		—	0 ·0324
	7 ·6908		—	0 ·0354
2	5 ·1381	Near asphyxia	0 ·0575	
	9 ·7735		—	0 ·0269
	5 ·4656		—	0 ·0404
3	7 ·0302	Between vanishing of reflexes and asphyxia	—	0 ·0333
	9 ·5823		—	0 ·0236

2. *Experiment in which Blood was defibrinated by shaking with Mercury before the addition of Alcohol.*

Only one determination was made by this method, since it might be objected that some chloroform was lost in the shaking process.

Table IV.

Number of experiment.	Weight of blood taken.	State of anæsthesia.	Chloroform by Nicloux.
	grammes.		
4	9 ·4017	After 55 minutes' inhalation of 2 per cent. chloroform	0 ·021
	5 ·9302		0 ·025

3. In this series of experiments a comparison was made, using oxalated and clotted blood.

Table V.

Number of experiment.	Weight of blood.	Condition of the blood.	State of anæsthesia.	Chloroform by Carius.	Chloroform by Nicloux.
	grammes.				
5	5·0748	—	Between disappearance of reflexes and asphyxia	0·0298	
	7·02	Oxalated ……	—	—	0·0237
	5·5657	,, ……	—	—	0·0244
6	5·2192	—	After 47 minutes with 3·7 per cent. chloroform	0·034	
	3·616	—		0·0358	
	6·883	Oxalated ……	—	—	0·0285
	8·2588	,, ……	—	—	0·0256
	10·97	Clotted………	—	—	0·017
7	4·6723	—	After 40 minutes' inhalation of 2 per cent. chloroform	0·0256	
	8·2447	Oxalated ……	—	—	0·022
	6·3256	,, ……	—	—	0·0248
8	4·5388	—	After 35 minutes' inhalation of 2 per cent. chloroform	0·016	
	7·0821	Oxalated ……	—	—	0·0235
	7·7917	,, ……	—	—	0·0206
9	4·1422	—	After half an hour inhalation of 3 per cent. chloroform	0·0447	
	7·9383	Oxalated ……	—	—	0·0338
10	4·2004	—	After a prolonged anæsthesia. Chloroform, given intermittently, about 3 per cent.	0·0485	
	6·6885	Clotted and clot broken up with glass rod	—	—	0·0333
	5·9814	Oxalated ……	—	—	0·031
11	4·2491	—	—	0·0354	
	5·2041	—	—	0·0339	
	11·6862	Clotted………	—	—	0·0236
	5·7553	,, ………	—	—	0·03
	9·4907	Oxalated ……	—	—	0·027

4. In the last series of experiments the cat was treated with hirudin in such proportion as to render the blood incoagulable for some minutes. We find that this is an excellent substance for checking the tendency to clotting, which is so liable to occur in cats at the moment of asphyxia.

Table VI.

Number of experiment.	Weight of blood.	Chloroform by Carius.	Chloroform by Nicloux.
	grammes.		
12	4·579	0·0319	
	4·1432	0·0327	
	14·9269	—	0·0334
	13·879	—	0·0359

Conclusions.

1. The method of chloroform determination, based on Dumas' reaction, though not possessing a very high degree of precision as an exact chemical method, as is apparent from a comparison of Tables I and II, is sufficiently accurate for most practical purposes and, as used by Dr. Nicloux, is a simple and rapid method, and one capable of giving satisfactory results for the estimation of small quantities of chloroform in air and in simple solution in such liquids as urine, etc.

2. In the case of the blood of anæsthetised animals, if the blood for analysis is allowed to clot before admixture with acid alcohol, the results by Nicloux' method are uniformly too low. An inspection of Tables III and V will show that results concordant amongst themselves are obtained when sensibly equal quantities of blood are taken, but, as might be expected, the figures decrease the larger the amount of blood used. In cases where smaller amounts of blood are taken and, consequently, the amounts of chloroform dealt with are very small, the results are often near the truth; but this, we think, is largely due to the inherent difficulty in the method as regards the determination of the end-point.

3. In cases where the clotting of blood was prevented by oxalate, the results more nearly approach those given by the methods we have previously adopted, but still are somewhat lower.

If we take the results given in Table V, the average percentage of chloroform indicated by our method is 0·0319, and by that of Nicloux in the case of oxalated blood is 0·0259. In our method we are satisfied that the average maximum error is not more than 5 per cent., generally considerably less. We find the difference between the results given by our method and that of Nicloux' method averages 19 per cent.

4. In the single experiment quoted with hirudin, the figures by the two methods are remarkably concordant.

5. On account of the variable and low results obtained with clotted blood, it may be inferred that the method of Nicloux may give low results when applied to organs or tissues which do not disintegrate when boiled with acid alcohol. We take this opportunity of stating that the expenses of the foregoing work were defrayed out of a grant made by the Royal Society.

Cyanogenesis in Plants. Part VI.—On Phaseolunatin and the Associated Enzymes in Flax, Cassava, and the "Lima Bean."

By Professor Wyndham R. Dunstan, M.A., LL.D., F.R.S., T. A. Henry, D.Sc. (Lond.), and S. J. M. Auld, Ph.D. (Würzburg).

(Received February 27,—Read February 28, 1907.)

In a previous paper of this series* it has been shown that the production of prussic acid by the seeds or beans ("Lima beans") of *Phaseolus lunatus* is due to the interaction of a cyanogenetic glucoside, phaseolunatin, with an enzyme, both these substances being proved to exist in the seeds.

Phaseolunatin was proved to have the composition and constitution of a dextrose ether of acetonecyanohydrin, but it was not then obtained in sufficient quantity to ascertain precisely the structure of the dextrose residue in the glucoside. Recently, however,† large supplies of "Java beans" (the beans produced by *Phaseolus lunatus* grown in Java) have been imported into this country, and we are indebted to Dr. Bernard Dyer for a small consignment of these beans, which has constituted the raw material from which the considerable quantities of phaseolunatin required in the course of the present investigation have been prepared.

Since the publication of our previous paper, it has been asserted by Kohn-Abrest‡ that these "Java beans" contain not one, but several cyanogenetic glucosides, and that none of these yield acetone on hydrolysis by hot dilute mineral acids or by the glucosidolytic enzymes present in the beans.

We have considered it necessary, therefore, to examine carefully the glucosidic product obtained from "Java beans" by the process originally used by us§ in the investigation of the beans of *Phaseolus lunatus* obtained from Mauritius and we have been unable to detect the presence of any other cyanogenetic glucoside in Java beans except phaseolunatin, identical in all

* Dunstan and Henry, 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

† 'Bulletin of the Imperial Institute,' 1905, vol. 3, p. 373, and 1906, vol. 4, p. 329.

‡ 'Comptes rendus,' 1906, vol. 143, p. 182.

§ Dunstan and Henry, *loc. cit.*

respects with that first isolated by us from the practically wild *Phaseolus lunatus* of Mauritius.* We have found no difficulty in confirming our previous observation that this glucoside yields acetone on hydrolysis, and is, in fact, a dextrose ether of acetonecyanohydrin.

We have shown recently† that the linamarin first isolated by Jorissen and Hairs from flax (linseed) is identical with phaseolunatin, and that the latter also occurs in the cassava of tropical countries. This identification of linamarin with phaseolunatin makes it possible to discuss the divergent views, which have been held by various investigators regarding the nature of the enzyme, which occurs in association with phaseolunatin in flax and in the beans of *Phaseolus lunatus*.

Jorissen, in his first paper‡ dealing with the production of prussic acid by flax, stated that the seed contained a substance on which emulsin acted in the same way as on amygdalin. In a later paper, Jorissen and Hairs§ recorded the isolation of linamarin (phaseolunatin) from flax and stated that it was decomposed by the enzyme associated with it in flax seed, but not by the emulsin of almonds. Subsequently linamarin was re-examined by Jouck,‖ who stated that it was decomposed by emulsin. In the third paper of the present series,¶ dealing with the isolation of phaseolunatin, the identity of which with linamarin had not then been established, it was stated that this glucoside was decomposed by emulsin, and that as the range of activity of the enzyme, occurring with phaseolunatin in the beans of *Phaseolus lunatus*, appeared to be identical with that of the emulsin of almonds, it might be assumed provisionally that these beans contained emulsin.

This statement was based on the result of a single experiment, in which prussic acid was undoubtedly liberated when a commercial emulsin preparation was added to an aqueous solution of the glucoside. This experiment has, however, been repeated frequently, using several different commercial emulsin preparations as well as emulsin prepared by ourselves from sweet almonds, and in no case have we been able to observe again the formation of prussic acid within a reasonable time, though in every case prussic acid was liberated on the further addition of the mixture of enzymes prepared from the beans of *Phaseolus lunatus* or from flax or cassava.

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* M. Kohn-Abrest's results are dealt with more fully in a recent paper (Dunstan and Henry, 'Annales de Chimie et de Physique,' January, 1907).

† Dunstan, Henry, and Auld, 'Roy. Soc. Proc.,' 1906, vol. 78, p. 145.

‡ Jorissen, 'Bull. Acad. Roy. Belg.,' 1884 (iii), vol. 6, p. 718.

§ Jorissen and Hairs, 'Bull. Acad. Roy. Belg.' (iii), vol. 21, p. 529.

‖ Jouck, 'Beiträge zur Kenntnis der Blausäure abspaltenden Glycoside,' Strassburg, 1902.

¶ Dunstan and Henry, 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

The statement that phaseolunatin is decomposed by the emulsin of almonds must therefore be withdrawn. It is probable that in the cases (recorded by Jorissen,* Jouck,† and in the third paper of this series‡) in which the liberation of prussic acid from phaseolunatin has been attributed to the action of emulsin the decomposition was in reality due to some secondary cause and not to the direct agency of emulsin.

There is, therefore, the fundamental difference between the emulsin of almonds and the enzymes occurring in *Phaseolus lunatus* beans, flax, and cassava, that whilst emulsin decomposes amygdalin and salicin and is without action on phaseolunatin, the enzymes of *Phaseolus lunatus,* flax, and cassava decompose all three of these glucosides. The question at once arises as to whether the enzyme associated with phaseolunatin in these three plants is merely an enzyme of the emulsin type having a greater range of activity, or is a mixture of emulsin with a second enzyme, and that the decomposition of phaseolunatin is due to the action of the latter. The first of these hypotheses seems at first sight the more plausible, since it is difficult to admit that the same mixture of two enzymes should occur in the three plants already mentioned, especially since none of them contains, so far as has been ascertained, any glucoside or complex sugar which could be attacked by emulsin. The further evidence we have obtained, however, leaves no doubt that, accepting present theories of enzyme action, these three plants do contain two enzymes, one of the emulsin type and the other of the maltase type.§ It is probable that to the action of the "maltase" the decomposition of phaseolunatin is due.

Fischer|| has shown that the glucosidolytic enzymes so far systematically examined are divisible into two classes, the one capable of decomposing the *α*-alkyl ethers of the hexoses and the other the stereo-isomeric *β*-alkyl ethers of these sugars. The first class of enzymes may be regarded as typified by the maltase of yeast and the second by the emulsin of almonds.

It is obviously possible from Fischer's generalisation to classify an

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* Jorissen, *loc. cit.*

† Jouck, *loc. cit.*

‡ Dunstan and Henry, *loc. cit.*

§ Since the present investigation was concluded, some results have been published by Marino and Fiorentino ('Gaz. Chim. Ital.,' 1906, vol. 36, p. 395), which seem to indicate that the maltase of malt is capable of decomposing both *α*- and *β*-glucosides; thus, according to these authors, it effects the decomposition both of maltose and salicin. They reject the obvious and simple explanation of these anomalous results, viz., that the maltase they used contained emulsin, on what appear to be insufficient grounds. If their conclusions were established, our present views respecting the nature and mode of action of the group of glucosidolytic enzymes would require to be considerably modified.

|| 'Zeit. Physiol. Chem.,' 1898, vol. 26, p. 75.

unknown glucosidolytic enzyme by ascertaining whether it is active towards the α-alkyl ethers of the hexoses or towards the stereo-isomeric β-ethers. E. F. Armstrong* has extended Fischer's work in this direction by showing that when the α-alkyl ethers of the hexoses are decomposed hydrolytically by enzymes of the maltase type the sugars immediately liberated are the α-forms of the hexoses, and that, similarly, when the stereo-isomeric β-ethers are decomposed by enzymes, they furnish the β-forms of the hexoses.

In both cases the forms of the hexoses first liberated in the solution change, gradually if left to themselves, or immediately if a trace of alkali be added to the liquid, producing the equilibrium mixtures of the α- and β-forms of the hexoses, thus exhibiting the phenomenon of mutarotation which Lowry† first explained in this way.

E. F. Armstrong‡ has extended these results to a number of the naturally occurring sugars and also to several of the synthetic glucosides, and has succeeded in showing that by the action of invertase on sucrose and raffinose the α-form of dextrose is produced and that emulsin decomposes β-methyl glucoside, liberating the β-form of dextrose.

Action of Yeast on Phaseolunatin.

Since it had proved impossible to decompose phaseolunatin by emulsin, which is an enzyme of the β-class, it seemed advisable to ascertain if it could be decomposed by yeast-maltase. The latter was prepared by mixing yeast, previously dried at 30° C., with sand and grinding the mixture with a small quantity of water in a mortar. The paste so obtained was set aside with more water for 24 hours and maintained at a temperature of 35° C., 1 per cent. of toluene being added as an antiseptic.

The aqueous extract so obtained was filtered through a porous tube of baked clay, and by this means a pale straw-coloured liquid was obtained free from yeast cells. This liquid readily decomposed α-methyl glucoside and phaseolunatin. The results of several of the experiments made are given in the following table:—

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* 'Chem. Soc. Journ.,' 1903, vol. 83, p. 1305.

† 'Chem. Soc. Journ.,' 1899, vol. 75, p. 213.

‡ *Loc. cit.*

Name of glucoside used.	Amount of maltase preparation used.	Time of action.	Amount of glucoside decomposed.
	c.c.	hours.	per cent.
A. α-Methyl glucoside, 0·2 gramme	20	25	59·0*
Phaseolunatin, 0·3 gramme ……	20	25	30·5†
B. Phaseolunatin, 0·3 gramme ……	30	20	22·5†
„ 0·3 gramme ……	30	30	30·0†
α-Methyl glucoside, 0·2 gramme	10	20	26·0*

* Determined by estimating the dextrose produced.
† „ „ „ prussic acid produced.

The action was found to proceed more readily, and could be more easily followed when, as suggested by Fischer,* dried yeast was substituted for the extract. Thus, in one particular case, using yeast dried at 30° C., 85 per cent. of the phaseolunatin was decomposed in 24 hours.

These results indicate that phaseolunatin is an α-glucoside, since it is decomposed by yeast-maltase. Attempts were therefore made to ascertain by experiment whether α-dextrose is produced initially when the glucoside is hydrolysed by an enzyme.

Nature of the Dextrose Residue in Phaseolunatin.

A preparation of the enzymes contained in the beans of *Phaseolus lunatus* was made by extracting the beans with cold water, exposing the extract in a vacuous desiccator over sticks of potassium hydroxide to remove the hydrocyanic acid formed and then pouring the aqueous extract into excess of alcohol. The precipitate so obtained was dried on a porous tile at the atmospheric temperature.

The characterisation of the sugar produced by the decomposition of phaseolunatin was carried out as follows. To a solution of 3 grammes of the glucoside in 20 c.c. of water, 0·5 gramme of the enzyme preparation was added and the mixture kept at 40° C. in a stoppered bottle.

For each observation a few cubic centimetres of the liquid were withdrawn, mixed with a small definite quantity of alumina cream and immediately filtered through a closely felted pad of asbestos. The optical rotation of the clear filtrate was then determined *before* and *after* the addition of a drop of ammonia solution, the latter being used as indicated by Lowry† to establish equilibrium. The same polarimeter tube was used throughout the experiments.

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* 'Berichte,' 1894, vol. 27, p. 1479.

† 'Chem. Soc. Journ.,' 1899, vol. 75, p. 213.

Series 1.—Temperature, 39° C.

Time of action.	Initial rotation.	Rotation after addition of ammonia.	Change in rotation.
hours.	° ′	° ′	° ′
0	−1 15	−1 15*	0 0
1	−0 46	−1 11	−0 25
2	−0 35	−0 58	−0 23
3	−0 34	−0 57	−0 23
20	−0 56	−0 56	0 0

* The non-occurrence of any immediate change in rotation indicates that the optical rotation of phaseolunatin is not affected by the addition of the minute quantity of alkali necessary in carrying out these experiments.

In this series of experiments 2·85 grammes of phaseolunatin were dissolved in 20 c.c. of water, and to the solution 0·5 gramme of the enzyme preparation was added.

Series 2.—Temperature, 40° C.

Time of action.	Initial rotation.	Rotation after addition of ammonia.	Change in rotation.
hours.	° ′	° ′	° ′
0·0	−1 0	−1 0	0 0
0·5	−0 44	−0 59	−0 15
1·5	−0 38	−0 55	−0 17
3·0	−0 29	−0 50	−0 21
20·0	−0 50	−0 50	0 0

The various portions of the liquid resulting from these two series of experiments were mixed and boiled with dilute sulphuric acid to ensure complete decomposition of the glucoside. The acid liquid was then neutralised with barium carbonate, evaporated to dryness on the water bath, and the residue extracted with strong alcohol. This extract was then decolourised with animal charcoal, and the contained sugar precipitated by adding excess of ether. The sticky precipitate was dissolved in diluted alcohol, from which on standing the sugar separated in the form of minute colourless plates, melting at 85°, which is the melting point of dextrose. It had, moreover, the same specific rotation as dextrose. It was shown in the previous paper* that the sugar produced by the hydrolysis of phaseolunatin is dextro-rotatory, and yields a phenylglucosazone, melting at 208°. The sugar produced by the hydrolysis of phaseolunatin is therefore dextrose.

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* Dunstan and Henry, 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

Further, the tabulated results given above show that an increase in the lævo-rotatory power of a solution of phaseolunatin, partially hydrolysed by the natural enzyme, is produced by the addition of ammonia. This can only mean that a highly dextro-rotatory substance initially produced by the action of the enzyme is transformed by the ammonia into one of lower dextro-rotatory power.

It will be seen that, taking the following data into account,

Specific rotation of		phaseolunatin	−27°·4
”	”	α-dextrose	+105°
”	”	β-dextrose	+22°
”	”	the equilibrium mixture of α+β-dextrose	+52°·5

these results can only be explained on the assumption that the sugar initially produced by the decomposition of phaseolunatin is α-dextrose, and that the glucoside itself is the α-dextrose ether of acetonecyanohydrin. Phaseolunatin appears to be the only naturally occurring α-glucoside so far known, all the other glucosides so far examined yielding the β-forms of the sugars on complete hydrolysis by enzymes.

Nature of the Glucosidolytic Enzymes present in Phaseolus lunatus.

Since α-dextrose is produced from phaseolunatin by the enzyme preparation obtained as already described from the beans of *Phaseolus lunatus*, it must be assumed that this preparation contains an α-enzyme and, since the glucoside is also decomposed by the maltase of yeast, it would appear that this α-enzyme may be identical with yeast maltase, or is at any rate of the same type.

The only other possible view is that the α-enzyme of *Phaseolus lunatus* may be identical with the invertase of yeast, but Fischer has shown that the latter does not decompose α-methyl glucoside, and we have found that yeast washings, containing invertase, have no action on phaseolunatin. In these two respects, therefore, invertase differs from the α-enzyme of *Phaseolus lunatus.*

The identity of the α-enzyme of *Phaseolus lunatus* with yeast maltase cannot, however, be definitely established, since, although the range of activity of the two enzymes appears to be similar, yeast maltase decomposes α-methyl glucoside and maltose more rapidly than does the α-enzyme of *Phaseolus lunatus*, and the latter hydrolyses phaseolunatin more quickly than yeast maltase does.

Since the enzyme preparation obtained from *Phaseolus lunatus* decomposes amygdalin and salicin, and in this respect resembles the emulsin of almonds, it must also contain a β-enzyme identical with or similar to emulsin. The only other possible hypothesis, viz., that *Phaseolus lunatus* contains a single glucosidolytic enzyme capable of decomposing both α- and β-glucosides, seems incompatible with our present views of the relationships of enzymes and glucosides.

A mixture of the same two enzymes must also occur in cassava and in flax* since, as we have shown previously, enzyme preparations obtained from these two sources behave in precisely the same manner as the enzyme preparation obtained from *Phaseolus lunatus.* It is also worthy of mention in this connection that yeast is capable of decomposing phaseolunatin, amygdalin, and salicin, and on this and other grounds has been assumed to contain an emulsin-like enzyme as well as maltase.†

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* Dunstan, Henry, and Auld, 'Roy. Soc. Proc.,' 1905, vol. 78, p. 145.

† Henry and Auld, 'Roy. Soc. Proc.,' 1905, vol. 76, p. 568.

Capillary Electrometer Records of the Electrical Changes during the Natural Beat of the Frog's Heart. (Preliminary Communication.)

By Francis Gotch, M.A., D.Sc., F.R.S.

(Received March 5,—Read March 14, 1907.)

It was shown, in 1880, by Burdon-Sanderson and Page, that when the ventricle of the excised frog's heart is connected with a capillary electrometer by two contacts, one lying on the base and the other on the apex, then every contraction of the heart was associated with electromotive changes of the following type. The electrometer meniscus was displaced in a given direction, the displacement then suddenly returned and, after a certain time, a second final displacement occurred in the opposite direction, which returned slowly; the recorded displacements are therefore of the type shown in fig. 1 of the annexed diagram. Most of these earlier experiments were carried out on the excised heart rendered motionless by a suitable ligature around the sino-auricular junction, and excited to activity by an induced current which was applied to the tissue, near one of the electrometer contacts. The observed effects were demonstrated to be due to the algebraic sum of an active process under one contact, of the propagation of this process to the tissue under the other contact, and of the occurrence of a similar active process at this further point. The two phases, such as occur in fig. 1, were further shown to be indicative of the time relations of these active processes. The electromotive change during the active process causes this tissue to become relatively negative as compared with the inactive tissue. This negativity commences under the proximal contact, nearest to the seat of excitation; its development is, however, cut short by a similar change occurring under the distal contact situated further off from the seat of excitation, thus giving the first electrometer displacement the character of a single spike. Whilst both changes are in full progress there is no electrical difference between the contacts, and thus an iso-electrical interval occurs; finally, the change at the proximal contact, having commenced first, ends first, and the distal change outlasting the proximal one, a terminal displacement of opposite sign is produced. All these well-known results have been supposed, with little experimental warrant, to occur in the beating frog's heart *in situ*, and a discrepancy thus exists between the assumed electromotive phenomena of the beating frog's heart and the electrical changes actually observed in the beating heart of mammals and of man. Numerous records have been obtained in man and mammals by making use of Waller's discovery that the electrometer, when appropriately connected with the surface of the body, shows the electromotive changes which accompany each beat of the heart *in situ.* These have been supplemented by observations upon the exposed beating heart (Bayliss, Starling, etc.). The latest records of this type in the case of man are those which Einthoven has obtained by the use of his delicate string galvanometer. All these records differ fundamentally from the records obtained by Burdon-Sanderson with the excised heart of both the frog and the tortoise, for, although the effects are diphasic, the second phase in the mammalian heart is of the same sign as the first (as in fig. 2), instead of being of opposite sign. It has thus been suggested that there is a want of parallelism between the activities of the mammalian heart and those of the heart in the frog and the tortoise.

The present experiments show that the changes observed when the frog's heart remains *in situ* and supplied by blood are precisely similar to those known to occur in the mammalian heart, they also bring out a further new point of no little interest, since, under certain conditions, a return wave of activity proceeding from the apex to the base is clearly demonstrable; this wave succeeds that which proceeds from the base to the apex. It is this return wave which is largely responsible for the extensive terminal phase of the whole electromotive effect in the beating heart.

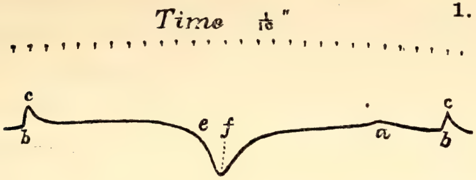
The observations now carried out have been made under a considerable variety of conditions, only a few of which can be at present referred to. The recording instrument employed was the extremely sensitive electrometer used by myself for the investigation of nerve. In order to indicate its sensitiveness, I may state that with one contact on a locally injured surface the full change under the other uninjured contact caused a very extensive displacement of the mercury meniscus which, when magnified by the lens used for its projection upon the recording photographic plate, displayed itself as a movement of from 150 to 180 mm.

The heart of the pithed frog was carefully exposed and left covered by pericardium; two contacts were then placed, one across the auriculo-ventricular groove, the other upon the ventricle apex. Each natural beat of the heart, thus left *in situ,* was associated with electromotive changes which caused electrometer movements of the character shown in fig. 2. In the right hand of this curve is the commencement of a heart beat, the rise of the curve indicating the relative negativity of the tissue under the contact upon the groove; the point (*a*) is the commencement of the auricular contraction, the point (*b*) the commencement of the ventricular contraction $0''{\cdot}46$ later. In the left part of fig. 2 the whole of the main ventricular change is displayed; it begins at the point (*b*) and commences to subside at the point (*f*). The successive events comprise an initial rise at (*b*) (negativity of the base), then a sudden fall at (*c*), occurring 0″·04 later; this gradually ceases, and after some little time a final or terminal rise (negativity of the base) recommences at (*e*). Such a record indicates that the base activity, commencing at (*b*) and beginning to subside at (*f*), is maintained longer than the apex activity which commences at (*c*) and begins to subside at (*e*); the duration of the base activity in this case was 1″·24, that of the apex activity was 1″·08. The curve is in all essentials similar in character to that already referred to as obtained from the mammalian heart *in situ*, both the first, or initial, phase and the second, or terminal, phase having the same sign. Similar records of less extent were obtained when the contacts were appropriately placed upon the external body wall of the frog without exposing the heart, whilst similar ones of larger extent were obtained when the pericardium was opened and the contacts placed upon the actual cardiac surface. If the heart is excised, then the records gradually change in character until ones of the character shown in fig. 1 may be obtained; these correspond in type with those described by Burdon-Sanderson as obtained from the immobile excised heart aroused by an artificial stimulus. It is, however, certain that the diphasic effect with phases of opposite sign, such as that shown in fig. 1, is not characteristic of the frog's heart when examined *in situ*, supplied with blood and beating naturally.

An interesting modification in the type of the records occurs if the ventricle is distended with blood; such distension is readily produced by slight pressure upon the vessels leading from the truncus arteriosus. In order to obtain the modification, the contact upon the groove is now so placed as to comprise that portion of the ventricle base which lies near the exit of the aorta. The modification is shown in fig. 3, which gives one full ventricular change. At the point (*b*) the base negativity due to activity of this part commences, the rise is cut short, as before, at the point (*c*), 0″·05 later, this being due to the propagation of the active state from the base to the apex and the commencement of the apex change; the apex change is, however, itself now cut short at the point (*d*) by a third change occurring about 0″·05 later, and this last change is of the same type as the initial base one, *i.e.*, indicates a second development of base negativity. On the subsidence of the apex effect, which begins at the point (*e*), these two more prolonged base effects, being in the same direction, are summated and produce the conspicuous terminal phase which culminates at the point (*f*). There is no doubt that the period from (*b*) to (*c*) indicates the time taken by the wave of activity in its propagation from the base to the apex, in this instance (temp. 12° C.) at a rate of from 130 to 140 mm. in 1 second; it follows that the period from (*c*) to (*d*) may be interpreted as the time taken by the propagation of the active state back again from the apex to that part of the base from which the aorta springs, in this instance also at a rate of from 130 to 140 mm. in 1 second.

Control experiments, with a contact upon the aortic bulb, showed that this second reversal was not due to the development of electromotive changes in the aortic wall or bulb, these being too feeble to affect the recording instrument. Moreover, although curves showing double reversals of the type shown in fig. 3 are obtained when the heart begins to get distended with blood, if, by further aortic compression, this distension becomes considerable, then the records tend to return to the type indicated in fig. 1, the ventricle base effect near the aorta being now almost imperceptible; on removing the distension and letting the blood out of the swollen heart by cutting through the bulb, curves of the type shown in fig. 3, with the double reversal of the first phase, are again obtained. It thus appears that adequate blood supply through intracardiac pressure brings into prominence the activity of that part of the ventricle which leads up to the aorta, and that this favourable influence, although interfered with by considerable distension, continues after the blood has left the heart; hence the new phenomena of double reversal are not directly caused by intraventricular blood pressure, but are indirectly associated with a previous adequate blood supply of the muscular tissue forming the ventricular wall. A number of experiments carried out with different positions of contacts and under a variety of conditions (local slight injury, local warming, etc.) have convinced me that the cause of the double reversal is the circumstance that, during the ventricular contraction, a wave of activity starts in that part of the base which is not immediately related to the aortic exit, and that this, having been propagated to the apex, then returns from the apex to the aortic part of the base.

Only one class of such experiments need be referred to in this preliminary communication. If contacts are placed on each lateral margin of the base near the groove, so that one lies upon the left side remote from the aorta, the other upon the right side near the spring of the aorta, then each natural beat of the heart is associated with electromotive effects of the type shown in fig. 4; these become more and more pronounced under the special conditions which favour the appearance of the double reversal. The curve given in fig. 4 shows the auricular beat commencing at the point (*a*); this is followed after an interval of 0″·45 by the base effect which begins at the point (*b*). Now, since the rise of the curve indicates relative negativity of the tissue under the contact on the left margin of the groove, which is remote from the aortic exit, it is clear that the change activity must commence here



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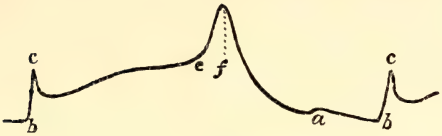

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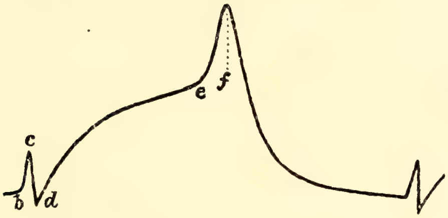

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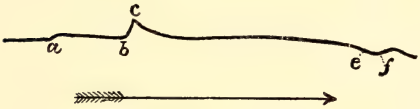

4.

DESCRIPTION OF FIGURES.

The curves given in the above figures are outlines of four photographic records of the displacement of the capillary electrometer meniscus. On each figure a rise in the curve indicates relative negativity of the tissue under a contact upon the auriculo-ventricular groove; all the curves are to be read from left to right.

FIG. 1.—Excised frog's heart, contacts upon auriculo-ventricular groove and upon the ventricle apex; natural beats; (*a*) auricular activity, (*b*) commencement of ventricular activity at base of ventricle, (*c*) commencement of ventricular activity at apex of ventricle, (*f*) summit of final ventricular phase.

FIG. 2.—Frog's heart *in situ*, contacts as in fig. 1; (*a*) commencement of auricular activity, (*b*) commencement of ventricular activity at base, (*c*) commencement of ventricular activity at apex, (*f*) summit of final phase.

FIG. 3.—Frog's heart *in situ*, but during or after distension with blood, contacts on ventricle base near spring of aorta and on ventricle apex; (*b*) commencement of general base activity, (*c*) commencement of ventricular activity at apex, (*d*) commencement of base activity localised to neighbourhood of aorta, (*f*) summit of final ventricular phase.

FIG. 4.—Frog's heart *in situ*, contacts on each side of base near groove, one on left side remote from aorta, the other on right side close to spring of aorta; (*a*) commencement of auricular activity, (*b*) commencement of activity under contact on left side of base remote from aorta, (*c*) commencement of activity on right side of base near aorta.

before a similar change commences under the other contact, that is, in the tissue near the aortic exit. The rise is, however, cut short at the point (*c*) by the commencement of such a change under the aortic base contact; this occurs in a number of measured records after a delay of from 0″·06 to 0″·08, *i.e.*, a longer interval than can be accounted for by the propagation time from base to apex. Moreover, the base change which commences first at (*b*), subsides first at (*e*), that commencing later at (*c*) subsiding later at (*f*), and thus causing a second or terminal phase of opposite sign to the first, or initial, one. Many records of this type have been obtained, and in every instance the first phase is of the same character; hence in the natural beat the whole base does not pass simultaneously into the active state with its associated relative negativity, the manifestation of such activity in the part near the exit of the aorta being always delayed.

The development of the frog's heart from a primitive **S**-shaped tube, and the well-known fact that a contraction of the wall of the aortic bulb occurs as the last stage of the whole cardiac cycle, are in accordance with the observations now described. These show that the frog's heart, when fully developed, continues to display signs of its ontogeny, and that even when these are structurally imperceptible, they can be revealed by appropriate physiological methods.

[The experiments here described were all carried out upon winter specimens of *Rana temporaria* during the months of January, February, and March.]

The Fermentation of Glucosides by Bacteria of the Typhoid-coli Group and the Acquisition of New Fermenting Powers by Bacillus dysenteriæ *and other Micro-organisms. Preliminary Communication.*

By F. W. Twort, M.R.C.S., L.R.C.P.

(Communicated by Leonard Hill, M.B., F.R.S. Received March 7,—
Read April 18, 1907.)

(From the Bacteriological Laboratory, London Hospital, E.)

For a number of years bacteriologists have largely relied upon certain fermentation reactions of sugars and alcohols for the differentiation of various micro-organisms which may otherwise be almost indistinguishable. This applies more especially to the case of bacteria occurring in the so-called "typhoid-coli group," although recently this method of differentiation has also been extended by Mervyn Gordon (1), Andrewes, Horder (2), and others, to the group of micro-organisms described under the general name of Streptococcus. As fresh fermentible substances have been introduced as tests, the number of different species occurring in such micro-organismal groups has become very large. Mervyn Gordon, in his studies on Streptococcus and Staphylococcus, introduced tests on certain sugars and upon such glucosides as coniferin and salicin, and he considered that these reactions were of help in differentiating different Streptococci from each other. MacConkey (3) has also published extended observations on the capability of certain lactose fermenting fæcal bacteria to attack sugars like saccharose and dulcite, and from the reactions obtained he was led to subdivide these lactose fermenters into four groups as follows:—

(1) A group represented by *Bacillus acidi lactici*, Hueppe, capable of fermenting lactose, but without action on dulcite or saccharose.

(2) A group, the typical representative of which is *Bacterium coli*, Escherich. In this case lactose and dulcite are fermented, but not saccharose.

(3) A group of the types *Bacillus neapolitanus*, Emmerich, and *Bacillus pneumoniæ*, Friedländer, containing organisms which ferment lactose, saccharose, and dulcite.

(4) A group containing micro-organisms capable of fermenting lactose and saccharose, but not dulcite. In this group were placed *Bacillus lactis aerogenes*, Escherich, *Bacillus capsulatus*, Pfeiffer, and *Bacillus cloacæ*, Jordan.

On utilising these sugar tests, MacConkey was able to differentiate such closely allied bacteria as *B. coli*, Escherich, *B. acidi lactici*, Hueppe, *B. lactis aerogenes*, Escherich, and *B. pneumoniæ*, Friedländer.

It was with a view to obtaining other fermentation tests which might be of service in separating organisms still remaining undifferentiated that the following experiments with glucosides were undertaken.

In carrying out these tests, an ordinary peptone water medium containing 2 per cent. of the glucoside to be tested was used. Such a medium was distributed in a number of test tubes, each of which contained a small inverted glass tube sealed at the upper end after the method recommended by Durham. In one series of experiments litmus solution was purposely left out of the medium to see whether any colour change might be observable in the glucoside under examination. In another series 5 per cent. of Kahlbaum's litmus solution was added, the medium being otherwise identical with the above. All the glucosides used were obtained from Merck.

In the case of insoluble glucosides an amount was added to the peptone water which would represent a 2-per-cent. solution.

All those media containing glucosides which by their colour obscured the reaction of the litmus solution were repeatedly tested on litmus paper during the growth of the organism.

Forty-nine glucosides were tested with the following 18 species of bacteria belonging to the typhoid-coli group :—

B. fæcalis alcaligenes, Petruschky.
B. dysenteriæ, Kruse.
„ Flexner (Pseudo-dysentery IV of Firth).
B. typhosus, Eberth-Gaffky.
B. paratyphosus, Brion and Kayser.
„ Schottmüller.
B. enteritidis, Gaertner.
B. paracoli, Widal.
B. coli, Escherich.
B. pneumoniæ, Friedländer (MacConkey strain).
„ „ (laboratory strain).
„ „ (Král strain).
B. neapolitanus, Emmerich.
B. pyogenes fœtidus, Passet.
B. acidi lactici, Hueppe.
B. lactis aerogenes, Escherich.
B. capsulatus, Pfeiffer.
B. cloacæ, Jordan.

At least six tubes of each glucoside medium were examined, the period of observation extending over four weeks.

Of the 49 glucosides examined, the following showed no reaction which could be ascribed to fermentation:—

Ononin.
Ericolin.
Digitalein.
Helleborein.
Cyclamin.
Apiin.
Hederaglucoside.
Tannin.
Jalapin.
Scammonin.
Colocynthin.
Plumierid.
Absynthin.
Quercitrin.
Hesperidin.
Convallarin.
Quabin.
Scopolin.
Frangulic acid.
Smilacin.
Condurangin.
Convolvulin.

The glucoside plumierid gave a dark green coloration with *B. capsulatus* and to a less extent with *B. lactis aerogenes*, *B. cloacæ*, and *B. pneumoniæ*, but no real fermentation was observed.

The remaining 27 glucosides showing some degree of fermentation with one or more of the test micro-organisms were as follows:—

Euonymin green.
Euonymin brown.
Iridin.
Senegin.
Coniferin.
Arbutin.
Salicin.
Syringin.
Quillajinic acid.
Periplocin.
Cathartinic acid.
Amygdalin.
Sapotoxin.
Saponin.
Bryonin.
Convallamarin.
Digitalin.
Strophanthin.
Populin.
Camellin.
Globularin.
Cerberid.
Baptisin.
Coronillin.
Gratiolin.
Adonidin.
Phloridzin.

In addition to the 18 test bacteria mentioned above, experiments were also carried out in the glucoside media with 26 other members of the typhoid-coli group. The latter cultures were isolated in the London Hospital from cases mostly of cystitis and colitis.

The results obtained with the whole 44 bacteria are set forth in the accompanying table, the type cultures are distinguished by an asterisk.

The sign + indicates a positive reaction, the sign — indicates that there was no change in the glucoside under observation. Vacant spaces indicate that no tests were made, the quantities of glucoside available being insufficient. "A" is used to denote acid formation; "G" gas formation.

In the table it can be seen that the bacteria have been subdivided into seven sub-groups according to their fermentative action on glucose, lactose, dulcite, and saccharose.

The last four of these sub-groups correspond to the four groups of MacConkey.

On looking at the table it is apparent—

1. That there is comparatively little difference between the fermenting capabilities of many of the lactose fermenters.

2. That two micro-organisms of a sub-group may differ in their ability to ferment glucosides, whereas micro-organisms in different sub-groups may present the same fermentative changes when tested on glucosides.

It will also be noted that the chief fermentations occur in the four sub-groups of lactose fermenters, but whereas the members of the true coli group give practically identical results when tested on glucosides, the results with members of the Friedländer group show greater variations, the chief of which is the inability of *B. neopolitanus* and Bacillus No. 41 to ferment salicin, arbutin, and syringin, which glucosides are easily fermented by *B. friedländer*. It must also be pointed out that the fermentation of many glucosides is neither marked nor constant.

Taking the individual glucosides in the table and reading from left to right, the following are the chief general characteristics of the fermentations:—

Brown euonymin.	Gas production very variable, often marked. Acid production seldom present, never marked.
Green euonymin.	Gas and acid formation never very marked.
Iridin and senegin.	Acid and gas extremely variable, often marked.
Coniferin, arbutin, salicin, syringin.	Usually show very distinct fermentation, both acid and gas being frequently very marked, occasionally slight.
Quillajinic acid.	Gas sometimes marked, acid always slight.
Populin.	Gas never excessive, acid production very variable.
Camellin, globularin, cerberid, periplocin, cathartinic acid.	Gas production always slight, acid never very pronounced.
Amygdalin.	Gas never formed, acid production variable and sometimes marked, especially with members of Capsulatus and Friedländer sub-groups.
Sapotoxin.	Gas production always slight, acid variable, but most pronounced, with Capsulatus and Friedländer sub-groups.
Saponin.	Gas very rarely present, acid usually slight.
Bryonin and convallamarin.	Acid and gas never very pronounced and very inconstant.

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Periplocin.
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Table showing the Action of Bacteria, belonging to the Typhoid-coli Group, on Various Glucosides.

No.		Euonymin (brown).		Euonymin (green).		Iridin.		Senegin.		Coniferin.		Arbutin.		Salicin.		Syringin.		Quillajinic acid.		Populin.		Camellin.		Globularin.		Cerberid.		Periplocin.		Cathartinic acid.		Amygdalin.		Sapotoxin.		Saponin.		Bryonin.		Convallamarin.		Digitalin.		Strophanthin.		Coronillin.		Gratiolin.		Baptisin.		Adonidin.		Phloridzin.		Glucose.		Lactose.		Dulcite.		Saccharose.		
		A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	
1*	*Bacillus fæcalis alcaligenes*, Petruschky (Conradi strain)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Sub-group I
2*	*Bacillus dysenteriæ*, Kruse (Král strain)	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	
3*	*Bacillus dysenteriæ*, Flexner (Firth strain)	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	
4*	*Bacillus typhosus*, Eberth (Danish strain)	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	
5*	*Bacillus paratyphosus*, Schottmüller (Král strain)	—	+	—	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	—	—	Sub-group II
6*	*Bacillus enteritidis*, Gaertner (Král strain)	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	—	—	
7*	*Bacillus paratyphosus*, Brion and Kayser (Král strain)	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	—	—	
8*	*Bacillus paracoli*, Widal (Král strain)	—	+	—	+	+	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	—	—	
9	Bacillus from urine—cystitis	—	—	—	—	+	—	—	—			—	—	—	—	—	—	—	—	+	—	—	+	+	—			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	—	—	—	—	+	+	Sub-group III
10	Bacillus from urine—cystitis	—	+	—	—	+	—	—	—			—	—	—	—	—	—	—	—	—	—	—	—	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	+	—	—	—	—	—	+	+	—	—	—	—	+	+	
11*	*Bacillus acidi lactici*, Hueppe (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	Sub-group IV
12	Bacillus from stool—colitis	—	+	+	—	+	+	+	—			+	+	+	+	+	+	+	+	+	—	—	+	—	+			—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	
13	Bacillus from stool—colitis	—	+	+	+	+	+	+	+			+	+	+	—	—	—	+	+	+	—	—	+	—	+			—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	
14	Bacillus from urine—cystitis	—	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	—	—	—	—	+			—	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	+	+	+	—	—	—	—	
15	Bacillus from urine—cystitis	—	+	+	+	+	+	+	—			+	+	+	+	+	+	+	+	+	+	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	
16	Bacillus from stool—colitis	—	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	—	—	+	+	+			+	—	—	—	+	—	—	—	—	—	+	+	+	—	+	—	+	—	—	—	+	—	—	—	+	—	—	—	+	+	+	+	—	—	—	—	
17*	*Bacillus coli*, Escherich (MacConkey strain)	—	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	Sub-group V
18	Bacillus from stool—colitis	—	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	—	+	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
19	Bacillus from stool—colitis	—	+	+	—	+	—	+	+			+	+	+	+	+	+	+	+	+	—	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
20	Bacillus from stool—colitis	—	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	—	+	—	—			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
21	Bacillus from stool—colitis	+	+	+	+	+	+	+	—			+	+	+	+	+	+	+	—	+	—	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
22	Bacillus from stool—colitis	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
23	Bacillus from urine—cystitis	—	+	+	—	+	+	+	+			+	+	+	+	+	+	+	—	+	—	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
24	Bacillus from urine—cystitis	—	+	+	—	+	+	+	—			+	+	+	+	+	+	+	—	+	—	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
25	Bacillus from urine—cystitis	—	+	+	+	+	+	+	—			+	+	+	+	+	+	+	—	+	—	—	+	—	+			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
26	Bacillus from stool—colitis	—	+	+	—	+	+	+	—			+	+	+	+	+	+	+	+	+	—	—	+	—	+			—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
27	Bacillus from urine—cystitis	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	—	+	+	—	+			—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
28	Bacillus from urine—cystitis	—	+	+	—	+	+	+	+			+	+	+	+	+	+	+	—	+	—	—	+	—	+			—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	
29*	*Bacillus pneumoniæ*, Friedländer (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—	+	+	—	+	—	+	+	—	+	+	+	+	+	+	—	—	—	—	+	+	—	+	+	+	—	—	—	—	+	—	+	+	+	+	+	+	+	+	Sub-group VI
30	Bacillus from stool—colitis	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	—	—	+	—	+	+	+	—	+	—	+	+	—	+	+	+	—	+	—	—	+	—	—	+	+			+	—	—	—	—	+	—	—	+	+	+	+	+	+	+	+	
31	Bacillus from stool—colitis	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—	+	—	+	+	—	+	+	+	—	+	+	—	—	+	+	+	+			+	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
32	Bacillus from stool—colitis	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	+	—	+	+	+	—	+	+	—	+	+	+	+	—			+	+	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
33	Bacillus from urine—cystitis	—	+	+	—	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—	—	+	—	+	+	—	+	+	+	—	+	+	—	+	+	+	+	—			+	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
34*	*Bacillus pneumoniæ*, Friedländer (laboratory strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—	+	—	—	+	+	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
35	Bacillus from sputum	+	+	+	—	+	+	+	+			+	—	+	+	+	+	+	+	+	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
36	Bacillus from stool—colitis	—	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	—	—	—	+	—	+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
37*	*Bacillus pneumoniæ*, Friedländer (Král strain)	—	+	+	—	+	+	+	—	+	—	+	—	+	—	+	—	+	+	—	—	—	—	—	—	+	—	—	—	—	—	+	—	+	—	+	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	—	+	—	+	—	
38	Bacillus from stool—colitis	+	+	+	+	+	+	+	+			+	—	+	—	+	—	+	+	+	—	—	+	+	+	+	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	+	—	—	—			—	—	—	—	+	—	—	—	+	+	+	+	+	+	+	+	
39*	*Bacillus pyogenes fœtidus*, Passet (MacConkey strain)	—	—	+	+	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	+	—	+	—	
40*	*Bacillus neapolitanus*, Emmerich (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	+	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
41	Bacillus from stool—colitis	+	+	+	+	+	+	+	—			—	—	—	—	—	—	+	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	
42*	*Bacillus capsulatus*, Pfeiffer (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	+	+	—	+	—	+	+	—	+	+	+	—	+	+	—	+	—	+	+	+	+	+	+	+	—	—	—	—	+	—	+	+	+	+	—	—	+	+	Sub-group VII
43*	*Bacillus lactis aerogenes*, Escherich (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	—	—	—	+	+	—	+	+	+	—	+	+	—	—	—	—	+	+	+	+	+	+	+	+	—	—	+	—	+	+	+	+	—	—	+	+	
44*	*Bacillus cloacæ*, Jordan (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	—	—	+	—	—	+	+	—	—	—	+	+	—	+	+	—	—	+	+	—	—	—	—	—	—	—	+	+	—	—	—	—	—	+	—	+	+	+	+	—	—	+	+	

(4149)

Digitalin and strophanthin.	Gas always small, acid production often well marked.
Coronillin, gratiolin, baptisin, adonidin.	Acid and gas usually slight and inconstant.
Phloridzin.	No gas production, acid formation slight.

Colour changes are observed in the case of certain glucosides, the most striking being the following.

Media containing *coniferin* often turn yellow or orange even although no apparent fermentation has taken place. Arbutin-containing media frequently turn brown when the cultures are old and alkaline. This is quite independent of any sign of fermentation.

Syringin media turn to a bright pink colour when fermented. In some instances, however, the colour is pale.

Amygdalin media turn lemon yellow in old alkaline cultures, showing no fermentative change.

Phloridzin media inoculated with *B. capsulatis*, *B. cloacæ*, *B. pneumoniæ*, *B. lactis aerogenes*, and Bacilli Nos. 30, 31, 32, 33, 38 and 41, show a bright brick red colour.

Plumierid becomes dark green in old alkaline cultures of *B. lactis aerogenes*, and the same may occur to a less extent with other micro-organisms.

The results quoted above seem to show that there is no sharp line of demarcation between the various sub-groups of lactose fermenting micro-organisms. It is true that nearly all the bacteria tested show slight individual characters, but they are so closely allied to each other that attempts to group them according to their lactose, dulcite, and saccharose fermenting powers must be regarded as artificial.

It seems, therefore, probable that the separate micro-organisms in the various sub-groups are not to be regarded as distinct species, but as varieties or hybrids of one or more species. If this be so, one might expect them to be constantly varying, losing old characters and gaining new ones according to the conditions under which they are grown, and it was with the object of testing this hypothesis that further series of experiments were undertaken. In the first place attempts were made to hybridise such organisms as *B. coli* and *B. typhosus*, but owing to technical difficulties no conclusive results were obtained.

Attempts were then made to produce variations in the pure cultures of the 18 test types of micro-organisms, the method employed being to grow each micro-organism for a succession of generations in a fluid medium containing a sugar which it had failed to ferment, the object being to see how far it could acquire a new fermenting property. After these experiments were practically finished, my attention was directed to a paper by Oscar Klotz (4), who had used the same method on one particular microbe of the coli group.

Klotz, however, only attempted to make his microbe regain characters which it had lost. In this he was successful after daily sub-cultures were made for about four generations, but it must be noted that his organism regained its fermentative powers even when grown on ordinary agar, although in this instance it was slower. The case with which Klotz was dealing was really one in which certain physiological functions were lost after the bacterium had passed through the body of an animal, these functions being regained in sub-cultures *in vitro.*

In the experiments which I have carried out the micro-organisms chosen had been grown in the laboratory for some years, their origin and properties being thoroughly well known. Many of them were obtained directly from Dr. MacConkey.

The media used in the experiments contained a sugar which the particular microbe is ordinarily unable to ferment. The sugar was added to ordinary salt peptone water to the extent of 2 per cent.

To ensure that the cultures were pure, they were first plated out, sub-cultures being obtained from single colonies. These sub-cultures were then passed through the usual sugar tests, being grown for four weeks at 37° C. Each culture so obtained and proved to be pure was transferred to a medium containing the sugar which at first it had failed to ferment. In such a medium it was allowed to grow for 14 days, when sub-cultures were again made to the same sugar medium and so on for successive generations.

All the sub-cultures were incubated for four weeks, the experiments being prolonged over months. The reason why the cultures were incubated for 14 days was to try to induce the microbe to attack the sugar after it had used up the other nutritive material in the medium. If division was still going on, it was assumed that any variety with a tendency to ferment the particular sugar would survive and multiply, since it could utilise the latter which the other members of the culture could not. In this way it might be expected that in successive generations a culture of increasing fermentative power would be obtained.

Such indeed proved to be the case; for several microbes were ultimately able to attack sugars which they, in accordance with universal bacteriological experience, at first failed to do. This change of function was slow, in most cases many successive generations being required before the new property was fully developed. Thus all the members of the para-typhoid sub-group were ultimately able to ferment saccharose. *B. typhosus* also acquired the property of fermenting dulcite and lactose.

The dysentery bacilli of Kruse and Flexner were ultimately able to ferment saccharose within 24 hours. *B. acidi lactici,* after several generations, fermented saccharose, and thus fell into the same sub-group as *B. lactis aerogenes. B. dysenteriæ* Kruse was also induced to ferment lactose, and so, fermenting glucose, saccharose, and lactose, came to be placed in the same sub-group with *B. lactis aerogenes,* and in this respect may be regarded as having fallen from the top of the typhoid-coli group to the bottom. Similar changes in the case of other micro-organisms ultimately led to the belief that division into distinct sub-groups separated by fermentation tests is impossible. The fermentation reactions are characterised by acid reactions, but are rarely accompanied by the production of gas. Special experiments were carried out with a typhoid bacillus which had acquired the power to ferment dulcite. When such a culture was plated out on agar, sub-cultures from single colonies retained the dulcite disruptive powers, although they were still capable of being agglutinated by a typhoid immune serum, thus proving that the fermentation was not due to any contaminating microbe. On inoculating the dulcite fermenting typhoid culture into a guinea-pig, sub-cultures were obtained showing the same reactions and these reactions were also maintained, even when the microbe was grown for several generations on ordinary peptone agar.

Conclusions.

1. A large number of glucosides may be fermented by many members of the typhoid-coli group of bacteria. The fermentations vary with the micro-organism tested, and the variations are as marked inside each sub-group of bacteria as between adjacent sub-groups.

2. The sugar-fermenting powers of an organism may be artificially changed by growing the said organism for a succession of generations in media containing a sugar which at the commencement of the experiment it was unable to ferment.

By this means a pathogenic organism may be altered until it gives fermentative reactions characteristic of a non-pathogenic member of its group. It is possible, indeed, that pathogenic organisms in the typhoid-coli group may so alter their characters that they become unrecognisable when growing for some time outside the body in soil, water, etc.

If this is so, it might partly account for the difficulty experienced in isolating *B. typhosus* from these situations.

It also seems possible that a non-pathogenic organism may lose its fermenting powers and become pathogenic should it find a suitable medium such as the alimentary canal, and regain its old characters when outside the body. This is, however, only a suggestion which at present is in no way proved.

In view of the results obtained with the typhoid-coli group of organisms, it seems quite possible that other organisms may show similar changes, and that the fermentation tests worked out by Mervyn Gordon for the Streptococci may also be inconstant if the same means of experimentation are employed.

In conclusion, I have to thank the Royal Society for the Government grants which enabled me to purchase the glucosides used in this research, and also Dr. MacConkey for many of the typical cultures used.

REFERENCES.

1. Mervyn Gordon, Thirty-third Annual Report of the Local Government Board, 1903—04, Supplement.
2. Andrewes and Horder, 'Lancet,' 1906, September 15, 22, and 29.
3. MacConkey, 'Journal of Hygiene,' 1905, vol. 5, p. 333.
4. Klotz, 'Journal of Infectious Diseases,' 1906, Suppl. No. 2, February.

On Reciprocal Innervation of Antagonistic Muscles.—Tenth Note.

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I. In previous notes* on this subject the examples of reciprocal innervation taken from the limb muscles have usually been from muscles acting at the knee. An advantage offered there is that the *vasti* and *crureus,* extensors of the knee, act directly on that one joint alone. A muscle acting on two joints is often an extensor of one of the joints and a flexor of the other. When such a muscle is thrown into contraction by reflex action, there may be a dilemma as to whether it is as an extensor or as a flexor that it is acting, and the answer will depend partly on the mode of fixation of the joints at the time. A double-joint muscle of this kind is *gastrocnemius*; it extends the ankle and flexes the knee. It is, therefore, of interest to examine how this muscle behaves when its antagonist, *tibialis anticus,* a flexor of the ankle, is thrown into contraction by reflex action.

Stimulation of any among various points of skin, or of the proximal end of various afferent nerves of the leg, excites reflex contraction of *tibialis anticus.* A nerve often used for the purpose in my observations has been the cutaneous branch of the peroneal nerve about a centimetre above the ankle (cat). In the reflex which then occurs in the limb, the following points can be made out. The ankle is flexed, and with the flexion of ankle occurs also flexion at knee and hip. If the anterior crural, obturator, hamstring, and popliteal nerve trunks, the insertions of iliopsoas, and psoas parvus, and the origin of pectineus muscles be severed, active flexion of knee and hip are excluded from the reflex. The active flexion at ankle still persists. On inspecting the tendons of the pretibial flexors of the ankle, that of *tibialis anticus* is seen to be drawn upward at each reflex, and with it also those of *extensor longus digitorum* and *peroneus longus,* but in my experience these latter are retracted less. If, in addition to the nerves and muscles mentioned above as severed, the peroneal nerve itself have all its muscular branches severed, but the branch from the popliteal nerve to the gastrocnemius be spared, and that muscle be exposed to inspection, usually no change in that muscle appears.

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* 'Roy. Soc. Proc.,' vols. 52, 66, 76, 77.

But if the conditions of experiment provide a pre-existent reflex contraction in the gastrocnemius, relaxation of it is seen to occur. A pre-existent reflex contraction of the gastrocnemius can be provided in several ways. I have for the purpose generally employed stimulation of the central end of the external plantar nerve of the opposite leg, *e.g.*, right. A light weight is attached to the left foot in such a way that the gastrocnemius acts against the weight in maintaining extension at the ankle. On stimulating the proximal end of the above-mentioned cutaneous branch of the left peroneal nerve, the ankle then flexes (fig. 1). The gastrocnemius being the only muscle of the leg whose innervation remains, this flexion must indicate reflex relaxation of that muscle. The requisite precurrent reflex contraction of the gastrocnemius may also be obtained by inducing decerebrate rigidity. One of the features of that rigidity is tonic extension of the ankle joint. Under this condition the reflex which produces contraction of the pretibial flexors of the ankle produces also, simultaneously, a relaxation of the gastrocnemius.

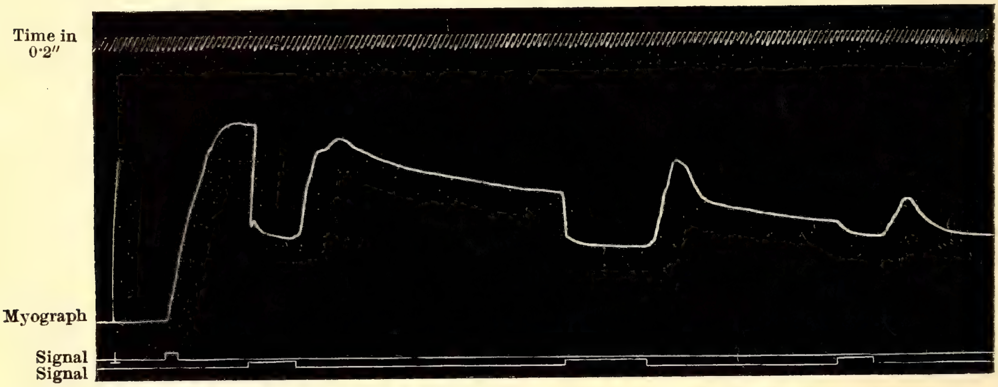

FIG. 1.—Myograph tracing from isolated right *gastrocnemius* (spinal cat). Upward movement of the recording lever registered contraction of the muscle, downward movement registered relaxation. The upper of the two signals below the myograph line marked the moment and duration of mechanical stimulation applied to central end of a cutaneous branch of the musculo-cutaneous division of left peroneal nerve. This stimulation caused reflex contraction of right gastrocnemius. The lower signal marked the times of application of weak faradism to central end of the similar cutaneous nerve of the right limb. In the right limb all other branches of the peroneal nerve, and all the branches of the popliteal except that to the gastrocnemius, had been severed, as well as obturator, anterior crural, and hamstring nerves, and the insertions of iliopsoas and psoas parvus, and the origins of adductor and pectineus muscles. The inhibitory relaxation of gastrocnemius is seen to be followed on cessation of the stimulation by marked after-rebound to contraction, especially after the second and third repetitions of the inhibitory stimulus. Time is marked above in fifths of seconds.

This reflex relaxation of *gastrocnemius* resembles that produced in the extensor muscles of the knee by stimulation of the proximal end of a cutaneous branch of the crural nerve. If, instead of the cutaneous branch of the peroneal nerve, the branch of the peroneal to the tibialis anticus muscle is severed, and then stimulated proximal to the section, other conditions remaining as above, the gastrocnemius similarly relaxes. In this case, therefore, stimulation of afferent nerve-fibres from the flexor muscle of the ankle evokes reflex relaxation of the extensor muscle of that joint. The case resembles, therefore, the reflex relaxation of the extensor of the knee evoked by stimulating the afferent fibres of the nerve of the flexor muscle of the knee.*

Let the conditions of experiment remain still as above except that the nerve of *extensor longus digitorum* is left unsevered, and let the tendons of that muscle at the ankle be severed to prevent their acting on the ankle joint. The *extensor longus digitorum* is seen to contract at the time when the gastrocnemius relaxes in the reflex excited from the central end of the nerve of *tibialis anticus.* And, conversely, stimulation of the central end of the nerve of *extensor longus digitorum* causes reflex contraction of *tibialis anticus*, together with concurrent relaxation of *gastrocnemius.*

II. In these instances, as in others previously reported,† stimulation of afferent nerve-fibres issuing from a muscle excites reflex contraction in that muscle and in muscles synergic with it, and, concurrently, causes reflex relaxation of the antagonistic muscles. Such instances are furnished by the nerves of *biceps cruris* (posterior part), *biceps brachii, gracilis, semi-tendinosus, tibialis anticus, extensor longus digitorum, peroneus longus*, etc. But it was shown‡ that if the nerve of the vasto-crureus muscle in the thigh be carefully split and one of its divisions be then severed, the central end of that division, when stimulated, provokes reflex relaxation of the vasto-crureus muscle itself, with concurrent reflex excitation of the antagonists of that muscle. Here the reflex exhibits, as in the other cases, reciprocal innervation of antagonistic muscles, but the case differs from the others in that that muscle whose nerve is stimulated is itself the field for relaxation, while, conversely, its antagonists, instead of being inhibited, contract. I find a parallel case to this is given by the nerve of *gastrocnemius.*

If all the nerves of the limb be severed except that of the outer head of gastrocnemius, and if then the central end of the nerve of the inner head of that muscle be stimulated, reflex relaxation of the outer part of the gastrocnemius occurs. If the nerves of *tibialis anticus* and *extensor longus digitorum* have not been severed, though their tendons at the ankle have been cut in order to prevent their mechanical action at that joint, reflex contraction of *tibialis anticus,* and, to a less extent, in my experience, of *extensor longus digitorum,* can, by inspection, easily be seen to accompany the reflex relaxation of the unparalysed part of the gastrocnemius. The inhibition can be demonstrated well by producing from the plantar nerve of the opposite leg a reflex contraction of the gastrocnemius just prior to exciting the reflex inhibition from its own nerve. The inhibition can also be demonstrated under decerebrate rigidity. The experiment can be varied by severing the nerve of the outer instead of the inner head of the gastrocnemius and keeping that of the inner head intact for revealing the effect on the muscle. The result is similar in the two cases. The nerve of *soleus* exhibits a similar reflex; so also in the fore limb do the nerves of *anconeus, supraspinatus,* and parts of deltoid and *triceps brachii* respectively. These likewise, while causing inhibitory relaxation of their own muscles, cause also reflex contraction in their antagonistic muscles, which flex respectively the elbow and the shoulder joints.

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* 'Roy. Soc. Proc.,' vol. 52.

† 'Roy. Soc. Proc.,' vol. 52.

‡ 'Roy. Soc. Proc.,' vol. 77.

III. Among afferent nerves proceeding from the muscles of the limbs, there are, therefore, as regards their reflex effects upon the musculature, two groups. Both of these evoke reciprocal innervation in antagonistic pairs of muscles; but one group evokes a reflex which excites that muscle from which the afferent nerve itself proceeds and concurrently inhibits the antagonistic muscles, while the other group evokes a reflex which relaxes the muscle from which the afferent nerve proceeds and concurrently excites the antagonistic muscles.

In both cases the reflex inhibition is not confined to one single muscle, but involves a number of muscles, just as the reflex contraction is not confined to one single muscle, but involves a number. Thus, with the inhibition of *gastrocnemius,* there can be seen to be associated, in one and the same reflex, reflex relaxation of *soleus, crureus, vastus lateralis,* and *vastus medialis.* Similarly, with the reflex contraction of *tibialis anticus* there can be seen to occur concurrent contraction, more or less pronounced, in *extensor longus digitorum, peroneus longus, biceps cruris* (posterior part), *semi-tendinosus, sartorius, tensor fasciæ femoris, gracilis, psoas, iliacus,* and *psoas parvus.* It is obvious, therefore, that the reflex inhibition is distributed over a group of synergic muscles, and that, similarly, the reflex contraction is distributed over another group of synergic muscles. The former are extensors of the ankle, knee, and hip; the latter are flexors of the ankle, knee, and hip.

In view of the synergic relation apparent in the muscles over which the reflex relaxation is distributed, it might be expected that stimulation of the afferent nerve of a muscle whose own nerve inhibits (reflexly) that muscle itself would also inhibit the other muscles whose nerves reflexly inhibit their own muscles. Experiment shows this to be actually the case. Thus the afferent nerve of *vasto-crureus* inhibits reflexly the gastrocnemius; the nerve of *gastrocnemius* inhibits reflexly the vasto-crureus; the nerve of *soleus* inhibits vasto-crureus, the nerve of *vasto-crureus* inhibits the soleus, and so on, so far as I have tested.

Similarly, stimulation of the afferent nerve proceeding from a muscle whose nerve throws its own muscle reflexly into contraction might be expected to excite reflex contraction in other muscles whose afferent nerves excite reflex contraction in their own muscles. Experiment finds this to be actually the case. Thus the nerve of *semi-tendinosus* excites, reflexly, contraction of the tibialis anticus; the nerve of *tibialis anticus* excites reflex contraction in the semi-tendinosus; the nerve of *extensor longus digitorum* excites reflex contraction in the tibialis anticus; the nerve of *tibialis anticus* excites reflex contraction in the extensor longus digitorum; the nerve of *psoas* excites contraction in the semi-tendinosus; the nerve of *semi-tendinosus* excites contraction in the psoas; the nerve of *psoas* excites contraction in the tibialis anticus; the nerve of *tibialis anticus* excites contraction in the psoas, and so on, so far as my experiments go.

IV. The reflexes elicitable from some of the limb nerves other than the above have also been observed in the experiments (cat). Among these additional nerves have been the following: the first dorsal digital, external plantar, internal plantar, the branch from the anterior tibial trunk to the foot (taken close above the ankle), the cutaneous branch of the musculo-cutaneous division of the peroneal nerve, the external saphenous nerve on the calf, the cutaneous branches of the sciatic given off above the popliteal space, the branch of the obturator to *pectineus*, the branch to *gracilis*, the branch to the adductor, the whole obturator nerve at the obturator foramen, the external cutaneous and the internal saphenous at various points along its course. These evoke reflex contraction in *psoas*, *semi-tendinosus*, *biceps cruris* (posterior part), *tibialis anticus*, *extensor longus digitorum*, *peroneus longus*—the reflex contraction in these two last is relatively slight, in my experience—*gracilis*, *sartorius*, *tensor fasciæ femoris*, *gluteus maximus* (anterior part), *gluteus minimus*, *pectineus*. The muscles, therefore, in which the reflex contraction has been observed to take place may be broadly described as those producing flexion of the limb, with some abduction and inward rotation. Consonantly with this, the reflex movement observed to result from stimulation of each of the afferent nerves, mentioned in the list at the outset of this section, has been flexion at knee, hip, and ankle, the flexion tending to be more marked at one or another of those joints according to circumstances, of which one has seemed to be the particular nerve chosen for excitation. These afferent nerves have also provoked reflex relaxation of *vasto-crureus, adductores, quadratus femoris, semi-membranosus, gastrocnemius,* and *soleus.* These reflex results have throughout the experiments been perfectly constant, in the sense that a nerve which provoked reflex contraction of a certain muscle never yielded reflex inhibition of that muscle, either in the same experiment or in another. The modes of stimulation of the nerves were, in each case, faradisation and mechanical compression separately employed for each nerve.

Of these afferent nerves examined, some are cutaneous, some deep, *e.g.*, issue from muscles, and some contain fibres from both cutaneous and deep sources. The reflex result of each was the same, in so far that each provoked contraction of the flexor muscles and reflex relaxation of the extensor muscles. I have shown previously* that the reflex movement provoked in the limb by faradisation of skin points in all parts of the surface, below the knee at least, is likewise flexion at knee, hip, and ankle. Putting these results together, it seems, therefore, that whereas in some reflexes, *e.g.*, the scratch reflex and the pinna reflex, the receptive field of the reflex is entirely cutaneous, in the flexion reflex of the limb the receptive field is both cutaneous and deep. For reasons adduced elsewhere,† the deep receptors of the body, *e.g.*, receptive end-organs in muscles, tendons, joints, bones, etc., can be conveniently classed together as *proprioceptive,* the immediate agent of stimulation in their case being usually not an object of the environment, but a part of the body itself. On the other hand, the receptors of the outer surface of the organism, *e.g.*, the skin, receive their stimulation usually more directly from the external environment, and have been termed *exteroceptive.*‡ The fields of reception of such reflexes as the scratch reflex and the pinna reflex are, therefore, purely exteroceptive; but the field of reception of the flexion reflex of the hind limb is both exteroceptive and proprioceptive. The same appears to be true, so far as my experiments go, of the flexion reflex of the fore limb, and also for the crossed extension reflex of the hind limb and the crossed extension reflex of the fore limb. The field of reception of each of these reflexes appears to be both exteroceptive and proprioceptive.

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* 'Journ. of Physiology,' vol. 34, p. 28; 'Integrative Action of the Nervous System,' London and New York, p. 127.

† 'Integrative Action of the Nervous System,' pp. 130, 336, etc.; 'Ergebnisse d. Physiologie,' p. 824, 1905.

‡ 'Integrative Action of the Nervous System,' *loc. cit.*; 'Ergebnisse d. Physiologie, *loc. cit.*

In the scratch reflex, the reflex, though from each point of skin in the receptive field a scratch reflex is produced, varies in certain characters as the locus of stimulation in the receptive field is shifted. The scratch reflex, considered as a generic type (the type reflex)* is, therefore, not really one reflex but a group of allied reflexes† differing somewhat one from another, yet presenting certain similar characters in common. The same is true of the flexion reflex of the limb. Although the movement is always flexion of the limb, the flexion tends to differ somewhat according as this or that skin-point, or this or that afferent nerve branch, is the seat of stimulation. These differences are more easily apparent in my experience under weak stimulation than under strong. The flexion reflex as a generic whole (the type reflex) is, therefore, not strictly one reflex, but is a group of allied reflexes, closely similar in general character, yet possessing individual differences one from another.

V. The crossed reflex excited in a hind limb by stimulation applied to the fellow limb of the opposite side exhibits, in my experience, a regularity almost as marked as does the uncrossed flexion reflex. The distribution of the effect seems mainly, if not entirely, to muscles affected by the uncrossed reflex. The crossed reflex has been elicited in my experiments by stimulation of the following afferent nerves: The plantar nerves, the anterior tibial close above the ankle, cutaneous branch of the musculo-cutaneous division of the peroneal, external saphenous, external cutaneous, internal saphenous at various heights up the limb, the nerves of *tibialis anticus, extensor longus digitorum, peroneus longus, p. brevis, p. quartidigiti, vasto-crureus, gracilis, pectineus, adductor*, and the larger nerve-trunks, such as obturator, peroneal, popliteal, and hamstring nerve. In the case of these nerves, the reflex effect observed in the crossed hind limb has been contraction of *crureus, vastus lateralis, vastus medialis, adductores, semi-membranosus, gastrocnemius*, and *soleus*. The resulting movement has been observed to be extension at knee, ankle, and hip. This movement is observed not only in the cat and dog but in the rabbit; though in the rabbit, in the ordinary progression of that animal, the hind limbs flex and extend synchronously usually instead of alternately as in cat and dog.

When *psoas, gracilis, semi-tendinosus, biceps cruris* (posterior part), *tensor fasciæ femoris*, and *extensor longus digitorum* are examined in the crossed reflex, they are, in my experience, not seen to contract. One might imagine that the reflex exerted no influence on them. But of this one cannot be sure until they have been thoroughly examined under conditions favourable for the detection of reflex inhibition. It seems not unlikely that they are inhibited in the crossed reflex.

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* 'Integrative Action of the Nervous System,' p. 127; 'Ergebnisse d. Physiologie,' *loc. cit.*, p. 822.

† 'Brit. Assoc. Reports,' 1904. Address to Sect. I.

It would seem therefore that, as said above, the distribution of the reflex effect is in the crossed reflex mainly, if not entirely, to the same muscles as those affected by the uncrossed reflex, but that the incidence of excitation and inhibition upon the motor neurones of those muscles is converse in the two reflexes. Those muscles which undergo reflex relaxation in the idiolateral reflex exhibit reflex contraction in the crossed reflex. In giving the above detailed lists of muscles observed to contract or relax in these reflexes, it is not intended to assert that the reflex throws into or out of contraction no other muscles of the limb. All that is meant is that in the muscles cited the reflex effect mentioned has been regularly observed.

VI. It seems noteworthy that those muscles, the afferent nerves issuing from which evoke reflex relaxation of those muscles themselves, possess certain physiological characters in common. They are all extensors, extending respectively the knee, the ankle, and the elbow. They are all muscles which, after decerebration of the animal (rabbit, cat, dog, monkey), markedly exhibit decerebrate rigidity. They all are muscles which, in the standing posture and in progression, counteract, by tonic contraction, the effect of gravitation and prevent the limbs yielding under the weight of the body. Two of them certainly yield "jerk-phenomena," *e.g.*, "knee-jerk" and "elbow-jerk." Another among them yields the allied phenomenon, ankle-clonus. They are all muscles for which the toxic agents, strychnine and tetanus-toxin, show marked predilection. They seem, therefore, in these respects to belong to a homogeneous functional group.

A question naturally arising in regard to these muscles is, whether in the afferent nerves issuing from them there exist no fibres at all which can provoke in them reflex contraction. In my experiments on their nerves the stimulus applied to the nerve trunk has been faradism or mechanical compression. It must be remembered that when stimulated in this way the plantar nerves likewise always evoke reflex inhibition in these muscles in the idiolateral hind limb. Yet there is a reflex—the "extensor thrust"*—obtainable from the *planta* (dog), which causes, not relaxation, but contraction of these very muscles; and this reflex ceases when the plantar nerves are severed. There appear, therefore, to be in the plantar nerves some afferent fibres which can excite reflex contraction of the extensor muscles of the limb, although when the whole of either plantar nerve trunk is excited by faradisation or by mechanical compression, the reflex produced is, in my experience, never contraction of these muscles, but is inhibitory relaxation. Similarly, therefore, with the afferent nerves issuing from these muscles, it may be that they contain some fibres which, if stimulated by themselves apart from the other fibres in the nerves, would evoke reflex contraction of the muscles, although the whole collection of nerve-fibres in the nerve-branch causes reflex inhibition of the muscles when the mode of stimulation is faradisation or mechanical compression of the nerve. This possibility is emphasised by other facts. These may be briefly stated as follows :—

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* 'Roy. Soc. Proc.,' vol. 66, p. 66; also 'Journ. of Physiology,' vol. 30, p. 39.

1. The tonic rigidity of *vasto-crureus* in decerebrate rigidity appears to be reflex, and the afferent channel of the reflex seems to lie in the nerve issuing from *vasto-crureus* itself. The tonic rigidity of this muscle is maintained when all the nerves of both hind limbs have been severed, excepting only the nerve of the vasto-crureus itself. The rigidity of the muscle immediately ceases on severance of the spinal afferent roots through which pass the afferent fibres of the vasto-crureus nerve itself. Similarly, also, with the tonic contraction of *gastrocnemius* under decerebrate rigidity, and with that of *soleus*. The tonic contraction of these muscles ceases only on severance of the afferent fibres issuing from those muscles themselves.

2. When all the nerves of both hind limbs have been severed, excepting only the nerve of *vasto-crureus*, squeezing the lower end of the shaft of the femur, exposed from the outer side, after exsection of the middle portion of the length of the shaft, evokes contraction of the vasto-crureus, and this contraction becomes inelicitable on severing the afferent spinal roots of the limb. Similarly, when the popliteal nerve remains as well as the nerve of *vasto-crureus*, contraction of *vasto-crureus* is elicited by squeezing the shaft of the tibia, but the reaction ceases on severing the afferent spinal roots of the limb. Again, under decerebrate rigidity, when all the nerves of both hind limbs are severed, excepting only the nerves of *gastrocnemius* and *soleus* of one side, light tapping on the shaft of the tibia excites increase of the tonic contraction of *gastrocnemius* and *soleus*; and this persists after the shaft of the femur has been cut through, but ceases on severance of the afferent spinal roots of the limb.

3. If, under decerebrate rigidity, the animal being in the inverted posture, the hind limb is lightly lifted by the foot into a position of full extension at knee and the foot is then released so that the limb below the knee drops into flexion, the free fall is seen to be suddenly checked much before full flexion is reached. The impression given is that of a contraction reflexly evoked in the extensors of the knee. The reaction occurs when all nerves of both hind limbs have been severed, excepting only the nerve of the vasto-crureus under observation. It ceases when the afferent spinal roots of the

limb are severed. A similar thing is seen with *adductor minor,* and with *quadratus femoris* under appropriate conditions for their tension. Similarly, under decerebrate rigidity, the tonic contraction of the vasto-crureus is markedly increased by a few passive extensions and flexions of the knee; and this holds good when the only nerve unsevered in the two hind limbs is that of the vasto-crureus in question. Similarly, the tonic contraction of the gastrocnemius-soleus can be markedly increased by a few passive extensions and flexions of the ankle, and this holds when all nerves have been severed except those issuing from these muscles themselves; the effect ceases when the spinal afferent roots have been cut.

4. On lifting a dog in which spinal transection above the lumbar region has been performed, and the period of shock passed over, it is extremely frequent for strong reflex extension of both hind limbs to occur. Both limbs are simultaneously extended at knee and ankle. This does not occur if, when the animal is lifted, the hind limbs are prevented from dropping under their own weight. Passive support of the limbs, under the thighs for instance, precludes the reflex.

5. It was stated above that faradisation or mechanical compression of the nerve of the vasto-crureus provokes with regularity an inhibitory relaxation of that muscle and of soleus and gastrocnemius. Occasionally, however, though, in my experience, quite rarely, if the nerves of the limb be all of them severed, excepting only that of the vasto-crureus itself, and that nerve be split into two divisions, central stimulation of one division may provoke *slight* extension at the knee if the initial posture of the knee at the time be full flexion.* But the same stimulation provokes inhibitory relaxation of the extensor if the initial posture of the knee at the time be fair extension. This result seems to resemble some reflex results recorded by v. Uexküll in Invertebrata.†

6. Finally, it may be recalled that various observers regard "knee-jerk" and "ankle-clonus" as reflex reactions, in spite of the admitted brevity of the latent time of the former. The knee-jerk is obtainable in the spinal animal when all nerves of the two hind limbs have been severed, excepting only the nerve of the vasto-crureus muscle in which the "jerk" is being examined.‡ It then ceases at once on severing the afferent spinal roots, through which pass the afferent fibres of the nerve of that muscle. Again, under decerebrate rigidity, ankle-clonus is obtainable (cat) when all the nerves of both hind limbs have been severed, excepting only the nerve of

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* *Cf.* 'Roy. Soc. Proc.,' vol. 66, p. 66; and vol. 76, B, p. 291.

† 'Zeitschrift f. Biologie,' vol. 44; 'Ergebnisse d. Physiologie,' 1904, p. 1.

‡ 'Roy. Soc. Proc.,' vol. 52, p. 557.

gastrocnemius-soleus. The reaction then ceases on severing the afferent spinal roots, through which pass the afferent fibres from the nerves of those muscles.

7. It seems possible that the diphasic result described in the following section (Section VII) may likewise point to the admixture, in the afferent nerves stimulated, of fibres provocative of opposite reflex effects (reflex relaxation, reflex contraction) in the same muscle.

VII. When the isolated vasto-crureus muscle with its nerve intact, or, similarly, the isolated gastrocnemius, soleus, *supraspinatus* or triceps brachii are under examination for the reflex effect upon them of excitation of the afferent nerves of their own limb, the inhibitory relaxation provoked is, on cessation of the stimulus, very frequently followed at once by contraction (fig. 1). This I have noted before,* but in systematically working through the afferent nerves of the limb it is so usual a reaction as to demand further mention here. It occurs with mechanical as well as with electric stimulation. It occurs in reflexes excited from skin as well as in those excited from isolated nerves, both cutaneous and deep. It occurs in the fore-limb as well as in the hind-limb. It seems of central origin.

Similarly, the inhibitory relaxation of the hamstring muscles, *e.g.*, left, provoked by stimulation of the central end of a nerve, *e.g.*, vasto-crureus nerve or plantar nerve, of the crossed hind limb (right), is sometimes followed, on cessation of the stimulus, by an immediate rebound to contraction in those muscles. This may be well seen after the nerve of the corresponding extensors (left) of knee has been severed. A similar result can be obtained from an afferent nerve, *e.g.*, median, of the crossed forearm (right). The stimulation of the median, giving inhibitory relaxation of the hamstring muscles (left), is followed, on cessation of the stimulus, by an immediate rebound to contraction. Again, under decerebrate rigidity, when all the nerves of the hind limb (left) have been severed, excepting only the nerve of the vasto-crureus, the reflex contraction of the vasto-crureus excited by stimulation of a nerve, *e.g.*, musculo-cutaneous, of the crossed (right) forearm, is often followed, immediately on cessation of the stimulus, by a relaxation of that muscle far more complete than that which existed in it immediately prior to the reflex contraction. The phase of reflex contraction seems here to be immediately followed by a phase of reflex relaxation; just as, conversely, in other cases the phase of reflex relaxation is immediately followed by a phase of reflex contraction.

The after-contraction following the inhibition seems best obtained when the stimulus is not very strong, but appears more marked after moderate than after very weak stimuli. It seems favoured by shortness of duration of the stimulus, *e.g.*, a duration of half a second or a second seems more favourable than one of 4 seconds. It is seen under decerebrate rigidity as well as after spinal transection. The after-contraction in *vasto-crureus* or *gastrocnemius* often does not extend the knee or ankle to as great a degree as existed under the decerebrate rigidity, immediately before the reflex inhibition—the first effect of the stimulus—set in. Sometimes, however, the after-contraction actually exceeds the degree of contraction existent prior to the reflex inhibition (fig. 1). The after-contraction never, in my experience, sets in during the actual application of the stimulus, but follows as a rebound after its cessation. The rebound is evidently reflex, for it often involves the extensors of hip, knee, and ankle together.

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* 'Roy. Soc. Proc.,' vol. 77, B, p. 486.

The inference was drawn above that the reaction is central in its mechanism. That is also borne out by the fact that it is met with under direct stimulation of the cross-section of the spinal cord itself. Thus I have met it when faradising, by the unipolar method, the lateral column of the spinal cord at the 10th thoracic level in the dog. The 7th cervical segment had been exsected seven months previously. From three points in the left ventrolateral column flexion of left hip and knee was obtained as a conjoined movement, together with extension of the right knee. There followed regularly, on cessation of the stimulus, a marked after-extension of left hip and knee. This after-extension was immediately cut short on stimulating the previous point again. But the after-extension was frequently increased by stimulating the left dorsal column. The face of the spinal section stimulated was that distal to the transection made at the time to expose the cross area of the cord for direct excitation.

[*Addendum.*—In Section VI above, it was pointed out that the muscles reflexly inhibited in the instances of reciprocal innervation given possess certain physiological characters in common. Among these characters, one that was mentioned was their counteracting gravity by tonic contraction in the habitual posture of the body when erect, *e.g.*, in standing. An instance of reflex reciprocal innervation is, however, furnished by the hind limb (cat), in which there occurs inhibitory relaxation of muscles which in some respects can hardly be supposed to have the function of counteracting gravity in the ordinary erect posture, *e.g.*, standing. This instance is well exemplified when the afferent nerve chosen for exciting the reflex is either of the plantar nerves or the musculo-cutaneous branch of the peroneal below the knee. Stimulation of any of these nerves excites reflex contraction of the flexors of hip, knee, and ankle, and reflex inhibition of the extensors of hip, knee, and ankle. But the stimulation excites, in addition, *gluteus minimus* and the anterior part of *gluteus maximus.* These two muscles produce abduction and internal rotation of the thigh. Together with the reflex contraction thus excited in these two muscles, the stimulation provokes reflex inhibitory relaxation of *adductor minor*, *adductor major*, and *quadratus femoris.* Of these three muscles, the two first are adductors of the thigh, the two last are external rotators of the thigh; all three are extensors of the thigh. In this reflex, therefore, not only are extensors relaxed when flexors contract, but adductors are relaxed when abductors contract, and external rotators are relaxed when internal rotators contract. It would seem, therefore, in this case that, as in the case of reciprocal innervation of antagonistic lateral muscles of the eyeball, the reciprocal inhibition of the muscles occurs even where the muscles are not by their tonus engaged in counteracting gravity.—April 16, 1907.]

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Studies on Enzyme Action. IX.—*The Enzymes of Yeast: Amygdalase.*

By R. J. CALDWELL, B.Sc. (Lond.), Leathersellers' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, and S. L. COURTAULD, B.A.

(Communicated by Professor Armstrong, F.R.S. Received May 9,—Read May 23, 1907.)

[*International Catalogue of Scientific Literature.*

Author's title slip :—D. Q. R.
Subject slips :—
D 1850 Amygdalin, hydrolysis of, by amygdalase.
D 8014 } Amygdalase, a new enzyme in yeast.
Q 1240 }
R 1910 Yeast, new enzyme in (amygdalase).]

The number of the 'Gazzetta Chimica Italiana' published on February 12, 1907, contains a communication received by the editor on December 17, 1906, "Su le azioni idrolitiche diverse prodotto da un solo enzima," by L. Marino and G. Sericano,* in which the conclusion is arrived at that the resolution of amygdalin by an extract of yeast into glucose and amygdonitrileglucoside is conditioned not by the enzyme maltase but by invertase.

We have long been aware that maltase does not effect the hydrolysis of amygdalin and to this extent can confirm Marino and Sericano's observations; but the conclusion that the cyanophoric glucoside is hydrolysed by invertase is not in accordance with our observations, which show that a specific enzyme, not hitherto recognised as a constituent of yeast, is the active agent in separating the glucose: it will be convenient to speak of this enzyme as *amygdalase.*

Amygdalin is one of the few natural glucosides which give two molecules of glucose when hydrolysed, the other product being benzylidene cyanhydrol, which—in dilute solution—decomposes spontaneously into benzaldehyde and hydrogen cyanide. There is reason to suppose that the two molecules of glucose are attached to one another—that is to say, that amygdalin is derived from a biose.†

It was shown by Emil Fischer, in 1894,‡ that an extract of dried Frohberg yeast (a bottom yeast) is capable of resolving the biose section of the molecule alone, a true glucoside and glucose being formed:—

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* Vol. 37, pp. 45—51.

† Evidence has already been adduced in favour of the view that this biose is not maltose (Caldwell and Courtauld, 'Trans. Chem. Soc.,' 1907, vol. 91, p. 670).

‡ 'Deut. chem. Ges. Ber.', 1894, vol 27, p. 2989.

$$C_6H_5CH(CN)O{-}C_{12}H_{21}O_{10} + H_2O \rightarrow C_6H_5CH(CN)O{-}C_6H_{11}O_5 + C_6H_{12}O_6.$$

In his earlier paper, Fischer speaks of the action as due to "invertin," but afterwards attributed the hydrolysis to the enzyme present in yeast which hydrolyses maltose.

Fischer's investigation was carried out at a time when little was known of the specific character of the enzymes. In the light of later work—especially that of E. F. Armstrong—it was improbable that maltase was the agent, emulsin (although highly active towards amygdalin) being without action on maltose and methyl-α-glucoside, which are both easily resolved by maltase. Our experiments were instituted to clear up this discrepancy.

To prepare amygdonitrileglucoside, Fischer digested a mixture of amygdalin with yeast-extract at 35°. On repeating the experiment in accordance with his directions, using a dried English brewer's yeast, we were surprised to find that the action took place far less rapidly than Fischer's description had led us to suppose it would; on testing the efficiency of the yeast-extract with maltose it was found to be almost inactive: judging from the relative stability of amygdalin and maltose in presence of acids, it was to be expected that the maltose would have been more rapidly hydrolysed than the amygdalin.

On considering all the circumstances, it appeared that we had worked at a higher temperature (35°) than that at which maltase exerts its maximum activity according to E. F. Armstrong's observations. The question arose whether the inactivity of the extract were not a consequence of overheating: comparative experiments were made, therefore, in which extracts prepared at different temperatures were digested, each at several temperatures, with solutions containing equivalent quantities of amygdalin, maltose and methyl-α-glucoside. These led to the discovery that the action on amygdalin and also on methyl-α-glucoside persisted after heating the solutions to such a temperature that the action on maltose was at an end—consequently, that maltase was not the active agent. Similar experiments at higher temperatures afforded evidence that the enzyme which hydrolysed amygdalin could be destroyed without depriving the solution of its power of hydrolysing cane-sugar.

The proof was thus obtained of the existence of a specific enzyme capable of effecting the separation of glucose from amygdalin. Whether or no this enzyme also hydrolyses methyl-α-glucoside or whether besides maltase two different enzymes are present in the extract, one of which attacks amygdalin, the other methyl-α-glucoside, cannot be decided at present: the discovery of an *α-glucase* different from maltase, however, is of considerable interest as an indication of the existence of separate α-glucosidoclasts.

It may be pointed out that the disproportion observed by E. F. Armstrong* in the relative rates at which maltose and methyl-α-glucoside are hydrolysed by maltase and acids respectively was perhaps in a measure due to the fact that the "maltase" contained the two enzymes which are capable of hydrolysing the glucoside, but only one capable of acting on the maltose.

Method followed.—The experiments were carried out in small closed flasks, which were heated in a Hearson incubator; excepting pipettes and funnels, all the vessels used were of hard Jena glass. The top yeast used was a pale ale yeast, for which we were indebted to Mr. Julian Baker. The freshly pressed yeast was first well washed with water saturated with toluene and without loss of time thoroughly drained on a vacuum filter; it was then rapidly dried on bibulous paper, the drying being completed by exposure over sulphuric acid *in vacuo.* Professor Emil Fischer had the kindness, at Dr. E. F. Armstrong's request, to obtain for us a quantity of dried bottom yeast (*S. Cerevisiæ*, Race 12, Berlin), which was ostensibly similar to that which he had used.

To prepare the extract, one part of dried yeast was added to 20 parts of water and a little toluene; the mixture was then digested at the required temperature, being shaken at intervals; it was finally clarified by filtration. Usually, the liquor was in contact with the yeast during 4 to 6 hours, experiment having shown that nothing was gained by extending the period.

Solutions of the hydrolytes were prepared containing four-tenths of a gramme-molecular proportion per litre. Equal volumes of the solution (20 c.c.) and of the yeast extract, heated to the temperature at which hydrolysis was to be effected, were quickly mixed in a flask; a sample of 10 c.c. was at once withdrawn and, the enzyme having been destroyed by the addition of a single drop of concentrated sulphuric acid (or in the case of cane-sugar, a solution of caustic soda), 10 c.c. of a 10-per-cent. solution of sodic acetate was run in; the liquid was then heated to 80° to precipitate the dissolved protein as far as possible. Finally, the filtered solution was examined polarimetrically in a 20-mm. tube at 25°. Meanwhile, the main bulk of the mixture had been placed in the incubator, where it was kept at the required temperature; at intervals, usually after 24, 48 and 72 hours from the commencement of an experiment, 10-c.c. samples were withdrawn, and treated as before described.

The amount of the substance hydrolysed was estimated by dividing the observed change in rotatory power by that calculated to correspond to complete hydrolysis; as the total change exceeded 200′ in every case, the error in the polarimetric reading being not more than about 1′, the values found may be regarded as accurate to within about ½ per cent.

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* 'Roy. Soc. Proc.,' 1904, vol. 74, p. 193.

Table I.—Action of Extracts of Top Yeast (*S. Cerevisiæ*) on Solutions of Equimolecular Concentration at 25° and 35°.

Glucoside used.	Yeast extracted at	Hydrolysis effected at	Amount hydrolysed in		
			24 hours.	48 hours.	72 hours.
			per cent.	per cent.	per cent.
Amygdalin	25°	25°	15·4	23·3	29·6
	35	25	15·4	21·2	28·3
	25	35	18·3	32·5	50·8
	35	35	18·7	25·4	31·1
Maltose	25	25	72·3	93·8	100·0
	35	25	21·8	26·9	30·3
	25	35	65·5	72·6	69·9
	35	35	11·7	15·9	
Methyl-α-glucoside	25	25	77·7	100·0	
	35	25	45·3	60·5	
	25	35	72·8	86·8	
	35	35	42·9	44·3	

Table II.—Action of Extracts of Top Yeast prepared at Temperatures between 0° and 55° on Solutions of Equimolecular Concentrations.

Glucoside used.	Yeast extracted at	Hydrolysis effected at	Amount hydrolysed in		
			24 hours.	48 hours.	72 hours.
			per cent.	per cent.	per cent.
Amygdalin	0°	25°	4·8	13·4	17·4
	15	15	11·4	16·4	24·4
	15	25	16·9	24·5	38·0
	15° (extract heated at 45°—1 hour)	25	15·1	21·0	
	45	25	16·1	26·7	36·0
	55	25	racemised	racemised	racemised
Maltose	0	25	54·1	69·6	75·9
	15	15	57·9	68·0	
	15	25	70·0	78·6	80·4
	15° (extract heated at 45°—1 hour)	25	3·9	29·7	32·3
	45	25	1·8	2·7	
	55	25	0·0	0·0	0·0
Methyl-α-glucoside	0	25	49·0	65·6	74·9
	15	15	50·6	64·9	
	15° (extract heated at 45°—1 hour)	25	39·4	50·6	63·9
	55	25	0·0	0·0	0·0

Maltase not Effective in Hydrolysing Amygdalin.—The results recorded in Table I show that amygdalin is more readily hydrolysed at 35° than at 25°, at whichever temperature the extract be prepared. In the case of maltose, the higher temperature is prejudicial, whether prevailing during the extraction or the hydrolysis.

Results are recorded in Table II of experiments in which the yeast was extracted both at lower (0° and 15°) and at higher (45° and 55°) temperatures than those before mentioned. In the first place, it is obvious that 15° is a less effective temperature than 25° not only in the case of amygdalin but also in that of maltose, a smaller percentage being hydrolysed at the lower temperature. But the extract prepared at 15° was the most active, 0° being apparently an altogether unfavourable temperature. The results obtained with an extract prepared at 45° show that the activity towards maltose is almost non-existent whilst unimpaired towards amygdalin. Extracts prepared at 15° and then heated to 45° show considerably reduced activity towards maltose. Extracts prepared at 55° were destitute of activity towards the three glucosides.

The experiments made with bottom yeast (Table III) gave results which showed that this yeast was far less active towards amygdalin than the top

Table III.—Action of Extracts of Bottom Yeast (*S. Cerevisiæ*, Race 12, Berlin).

Glucoside used.	Yeast extracted at	Hydrolysis effected at	Amount hydrolysed in		
			24 hours.	48 hours.	72 hours.
			per cent.	per cent.	per cent.
Amygdalin	25°	35°	4·6	4·6	11·7
	35	25	6·2	6·2	7·5
	25	25	6·2	4·6	8·3
	15	25	3·3	4·6	5·8
	15° (extract heated at 50°—1 hour)	25	4·6	3·3	4·6
Maltose	25	35	67·8	83·6	86·9
	35	25	52·2	68·1	74·0
	25	25	59·7	74·3	81·2
	15	25	53·1	68·3	74·6
	15° (extract heated at 50°—1 hour)	25	15·5	21·8	26·0
Methyl α-glucoside	25	35	61·9	76·3	
	35	25	40·6	55·8	65·5
	25	25	59·0	75·1	82·7
	15	25	43·3	66·2	71·9
	15° (extract heated at 50°—1 hour)	25	22·5	34·1	35·3

yeast, although the two extracts were almost equally active in hydrolysing maltose. The maltase in the bottom yeast appears to have been somewhat more resistant, a considerable proportion surviving in the extract which had been heated to 50°. The extract prepared from this yeast at 25° was more active than that prepared at a higher or lower temperature, 15° being the most favourable temperature in the case of the English top yeast; moreover, the hydrolysis of maltose was best effected by this yeast at 35° instead of at 25°.

Results similar to those recorded in Table III were obtained with another sample of yeast reported to be a bottom yeast grown in maize wort.

In view of the high temperature at which it exerts its maximum fermentative power, it appeared desirable to examine the remarkable yeast *S. Thermantitonum* described by Grove Johnson. Mr. Johnson was good enough to give us some quantity of the yeast: unfortunately, the extract prepared from it was so highly coloured that satisfactory measurements could not be made with it. The impression was gained that it resembled that prepared from the bottom yeast and that possibly amygdalin was not attacked by it.

Results are recorded in Tables IV and V which finally led to the conclusion that maltase can be entirely destroyed by heating, whilst the extract retains its activity towards amygdalin almost unaltered. Owing to the greater stability of the maltase in the case of the bottom yeast, it was necessary to heat the extract during a longer period (three hours instead of one) in order to render it inactive towards maltose.

Table IV.—Action of Extract of Top Yeast prepared at 15°.

Glucoside used.	Treatment of extract.	Amount hydrolised at 25°.		
		24 hours.	48 hours.	72 hours.
		per cent.	per cent.	per cent.
Amygdalin	Unheated	13 ·9	21 ·7	28 ·3
	Heated at 45° (2 hours)	17 ·8	25 ·4	31 ·7
	„ 50° (1 hour)	18 ·3	25 ·8	34 ·6
Maltose	Unheated	65 ·8	76 ·4	79 ·1
	Heated at 45° (1 hour)	16 ·1	22 ·7	23 ·3
	„ 45° (2 hours)	1 ·5	1 ·8	1 ·8
	„ 50° (1 hour)	0 ·0	0 ·0	0 ·0
Methyl-α-glucoside	Unheated	60 ·9	71 ·1	75 ·1
	Heated at 45° (2 hours)	16 ·5	26 ·9	32 ·5
	„ 50° (1 hour)	15 ·0	23 ·7	29 ·3

Table V.—Action of Extracts of Bottom Yeast prepared at 25°.

Glucoside used.	Time of extraction.	Treatment of extract.	Amount hydrolysed at 25°.		
			24 hours.	48 hours.	72 hours.
	hours.		per cent.	per cent.	per cent.
Amygdalin	20	Unheated	7·9	15·4	
	4	,, ...	4·6	8·8	10·4
	4	Heated at 35° (3 hours)	3·8	7·1	8·8
	4	,, 50° ,,	2·9	5·0	5·0
Maltose	20	Unheated	71·0	82·1	
	4	,, ...	59·1	70·7	78·8
	4	Heated at 35° (3 hours)	59·1	73·4	81·5
	4	,, 50° ,,	0·0	1·4	1·2
Methyl-α-glucoside	20	Unheated	76·6	88·8	
	4	,, ...	55·8	71·1	80·3
	4	Heated at 35° (3 hours)	50·2	67·9	78·7
	4	,, 50° ,,	2·1	1·7	3·6

Non-identity of Invertase and "Amygdalase."—The observations (recorded in Table II) that amygdalin was not attacked by an extract which had been prepared at 55° not only led us to think that the hydrolysis of amygdalin by yeast was not conditioned by maltase, but also that invertase was not the active cause of change—inasmuch as it is well known that invertase is far from being destroyed at this temperature. Experiments which are recorded in Tables VI and VII were made to test this point directly. The results show that the assumption made by Marino and Sericano before referred to cannot be accepted. An extract which has been heated at 60° and is entirely without action on amygdalin and methyl-α-glucoside still preserves much of its activity towards cane-sugar.

Marino and Sericano removed the maltase from the mixture of enzymes derived from yeast by subjecting the extract to diffusion; they overlooked the possibility that the less diffusible fraction—which they proved to be free from maltase—contained any other enzyme than invertase.

It may be mentioned as a point of interest that bottom yeast, although equally rich in maltase, appears to contain a far smaller proportion of both invertase and amygdalase than top yeast. At the end of 24 hours only 2·7 per cent. of amygdalin and 91·5 of cane-sugar had been hydrolysed by an extract of bottom yeast as compared with 10·5 per cent. of amygdalin hydrolysed by an extract prepared under like conditions from top yeast, which also hydrolysed cane-sugar completely in the course of a much shorter period than 24 hours. The extreme activity of top yeast towards cane-sugar is clearly indicated by the results given in Table VII.

Table VI.—Action of an Extract of Top Yeast prepared at 25° before and after Heating at Higher Temperatures.

Glucoside used.	Treatment of extract.	Amount hydrolysed at 25°.		
		24 hours.	48 hours.	120 hours.
		per cent.	per cent.	per cent.
Amygdalin	Unheated	10·5	17·9	
	Heated at 50° (1 hour)	7·8	17·9	
	" 60° "	0·0	0·0	racemised
Cane-sugar	Unheated	100·0	100·0	
	Heated at 50° (1 hour)	100·0	100·0	
	" 60° "	54·2	86·8	100·0
Methyl-α-glucoside ...	Unheated	40·2	51·4	
	Heated at 50° (1 hour)	1·7	3·2	
	" 60° "	0·0	0·0	0·0

Table VII.—Action of an Extract of Top Yeast prepared at 25° before and after Heating at 60° during 1 hour.

Glucoside used.	Treatment of extract.	Amount hydrolysed at 25° in 52 hours.
		per cent.
Amygdalin	Unheated	27·5
	Heated at 60°.......................	0·0
	" 60° and acidified* ...	0·0
Cane-sugar	Unheated, diluted to 1 in 50 ...	96·6
	Heated at 60°.......................	100·0
	Heated at 60° and acidified	100·0
Methyl-α-glucoside	Heated at 60° and acidified	0·0

* The extract which had been heated at 60° became sufficiently alkaline to convert amygdalin into isoamygdalin after a long period of digestion (Table VI). N/1000 acetic acid does not condition any measurable amount of hydrolysis. The expression "acidified" in the table is to be interpreted as meaning that acetic acid was added in the proportion of 0·001 gramme-molecule per litre of extract.

Hydrolysis of Methyl-α-Glucoside.—It will be observed on reference to the tables, that methyl-α-glucoside is always more or less attacked so long as amygdalin is acted upon, but that a very marked diminution in activity towards this glucoside attends the disappearance of the maltase (Tables IV and V). The inference that the methyl-α-glucoside is attacked by the two enzymes maltase and "amygdalase" cannot well be disputed—it is clearly necessary to assume that either an α-glucase exists together with amygdalase, or that one and the same enzyme (amygdalase) is concerned in the hydrolysis of both amygdalin and methyl-α-glucoside.*

It is noteworthy that after the maltase is destroyed there is a coincident variation in the amount of amygdalin and methyl-α-glucoside which undergo hydrolysis in the case of different yeasts (Tables IV and V).

Moreover, a yeast extract in which the maltase has been destroyed produces an effect on a mixture of methyl-α-glucoside and amygdalin markedly smaller than the sum of its effects on the two compounds taken separately (Table VIII). As there is competition for the enzyme when two substances are hydrolysed together (*cf.* Henri),† this result would also seem to show that a single enzyme is active.

It has been stated by Ter Meulen‡ that only glucose retards the hydrolysis of amygdalin by emulsin and that maltose is without effect. Apparently maltose is without effect on the hydrolysis of amygdalin by amygdalase (Table VIII); and in this respect *galactose* and *lactose* are also inert, whilst glucose has a marked inhibitory effect.

It is impossible at present to identify amygdalase with any known enzyme. The enzyme is present in varying amounts in the different yeasts we have examined, and appears to be equally well extracted from the dried material at all temperatures from 15° to 45°. Whereas a low temperature of extraction, 0°, affords a very small proportion of active amygdalase (Table III), the quantity is increased by heating the extract for a short time at 45°; a similar effect is produced by heating an extract prepared at 15° (Table IV): that is to say, the amygdalase is originally dissolved as part of a more complex protein or zymogen molecule, which is hydrolysed at the higher temperatures. Proof of its stability is afforded by the fact that it is contained in invertase prepared by O'Sullivan and Thompson's method, which involves the autolysis of the yeast and precipitation of the enzyme by alcohol—a process which entirely destroys maltase.

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* Marino and Sericano speak of using 20 kilogrammes of yeast. After this had been washed and dried, it was extracted with water; the precipitate produced by adding alcohol to the extract was purified by dialysis. Eventually they obtained only 2·5 grammes of dried substance. They state definitely that their invertase was prepared by continuing the dialysis until methyl-α-glucoside was no longer affected. However, they give no details of the experiments which proved this to be the case and it is noteworthy that amygdalin appears to have been but slowly hydrolysed by their material, even when this was used in large proportion.

† 'C. R. Soc. Biol.,' 1903, vol. 55, p. 868.

‡ 'Rec. Trav. Chim.,' 1905, vol. 24, p. 444.

Table VIII.—Action of Amygdalase from Top Yeast on Amygdalin in presence of Maltose, Methyl-α-Glucoside, Glucose and Galactose at 25°. The extract was prepared at 15° and subsequently heated at 50° during two hours to kill the maltase.

Substance hydrolysed.	Rotatory power change at 25°.		
	24 hours.	48 hours.	72 hours.
Amygdalin alone	−0° 37′	−0° 58′	−1° 10′
Maltose alone	+0° 1′	+0° 0′	+0° 0′
Sum	−0° 36′	−0° 58′	−1° 10′
Amygdalin and maltose together	−0° 39′	−1° 0′	−1° 14′
Retardation	0° 3′	0° 2′	0° 4′
Amygdalin alone	−0° 37′	−0° 58′	−1° 10′
Methyl-α-glucoside alone	+0° 25′	+0° 42′	+0° 54′
Sum	−0° 12′	−0° 16′	−0° 16′
Amygdalin and methyl-α-glucoside together	−0° 18′	−0° 28′	−0° 40′
Retardation	0° 6′	0° 12′	0° 24′
Amount hydrolysed at 25°.			
	per cent.	per cent.	
Amygdalin alone	17·5	26·2	
Amygdalin and glucose together	10·4	24·1	
Amygdalin and galactose together	16·7	28·3	

Studies on Enzyme Action. X.—The Nature of Enzymes.*

By Henry E. Armstrong, F.R.S., and E. Frankland Armstrong.

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[*International Catalogue of Scientific Literature.*
Author's title slip :—D. Q.
Subject slips :—
D 1820 Cane sugar, hydrolysis by invertase.
D 8012, Q 1230 Enzymes, nature of (invertase, maltase).]

The study of the action of enzymes has now reached a stage at which it may be well, in view of their importance as vital agents, to consider what is established and to call attention to some of the principal issues which remain to be elucidated.

Specific Activity of Maltase and Emulsin.—As the investigation is extended, the evidence becomes more and more convincing that the action which an enzyme exercises is specific; in other words, that it is limited to compounds of a particular type—to a greater extent, indeed, than is recognised in earlier communications of this series, which in some few particulars need modification.

In No. III a table is given (p. 520) in which it is indicated (on the authority of Emil Fischer) that maltase hydrolyses α-galactosides as well as α-glucosides and that galactose has a slight retarding effect. At the time when the experiments to which the table relates were carried out, no opportunity had been found of verifying Fischer's statement. Subsequently, when experiments were made with methyl-α-galactoside, extracts from both top and bottom yeast proved to be entirely without action. It was then ascertained that the summarised account of Fischer's work which had been consulted† contained an incorrect statement and that the results we had obtained were in harmony with those recorded by Fischer in his original papers.‡

The slight retardation of the action of maltase by galactose did not appear surprising so long as it was supposed that galactosides were hydrolysed by the enzymes; when, however, this was found not to be the case, it became necessary to re-examine the action: ultimately, the retarding effect of galactose was traced to the presence of an alkaline impurity.*

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* *Former Communications.*—I, 'Chem. Soc. Trans.,' 1903, vol. 88, p. 1305; II, 'Roy. Soc. Proc.,' 1904, vol. 73, p. 500; III, *ibid.*, p. 516; IV, *ibid.*, p. 526; V, *ibid.*, 1904, vol. 74, p. 188; VI., *ibid.*, p. 195; VII, *ibid.*, 1905, B, vol. 76, p. 592; VIII, *ibid.*, p. 600; also *ibid.*, 1904, vol. 73, p. 537; 1906, B, vol. 78, p. 376.

† 'Zeit. Phys. Chem.,' 1898, vol. 26, pp. 66 and 69.

‡ 'Ber.,' 1894, vol. 27, p. 2987, and 1895, vol. 28, p. 1430.

In No. V, in which the action of emulsin on milk sugar is discussed, attention is called to the evidence in favour of the view that emulsin is capable of acting on β-galactosides (*i.e.*, milk sugar) as well as on β-glucosides; also to Bourquelot and Hérissey's contention that probably lactase is present in ordinary emulsin. Experiments to be described in a later communication, made specially with the object of clearing up this question, leave no doubt that the activity of emulsin *as ordinarily prepared* is due, as Bourquelot and Hérissey and also Pottevin have contended, to the presence of a distinct enzyme capable of affecting the β-galactoside, on which emulsin proper has no action.

To state our opinion in brief, maltase is capable of hydrolysing α-glucosides alone, whilst emulsin hydrolyses β-glucosides.

Correlation of Enzyme with Hydrolyte.—In view of these considerations, the suggestions should be cancelled which were put forward in the third communication† with reference to the extent to which configuration may be modified without nullifying the action of an enzyme.

Attention was directed in this communication to the evidence of a close correlation in configuration between enzyme and hydrolyte afforded by the controlling influence on the rate of change of a glucoside which the hexose derived from it exerts; welcome confirmation of this conclusion is afforded by Ter Meulen's observations.‡

A further series of experiments has been made with specially purified materials which enable us to confirm and extend the earlier observations; it is unnecessary to describe these in detail, as the method followed has already been described. The results are summarised in the following table:—

Enzyme.	Hydrolyte.	Inhibitants.	Destitute of inhibiting power.
Maltase	Maltose	Glucose, methyl-β-glucoside	Galactose, fructose, mannose, sorbose, arabinose, mannitol, dulcitol
Invertase	Cane sugar	Glucose, fructose...	Galactose, mannose, mannitol, dulcitol
Lactase	Milk sugar	Galactose, methyl-α-galactoside	Fructose, mannose, arabinose, xylose, mannitol

The evidence that enzyme and hydrolyte must be in complete correlation appears to be little short of absolute in the case of the sucroclasts.

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* *Cf.* Caldwell, 'Roy. Soc. Proc.,' 1906, vol. 78, p. 283.

† Vol. 73, p. 525.

‡ 'Rec. Trav. Chim.,' 1905, vol. 24, p. 444.

The Hydrolysis of Cane Sugar.—The evidence as to the manner in which the hydrolysis of cane sugar is affected by the products of change is conflicting. O'Sullivan and Tompson stated, in 1890,* that the products of inversion are without influence on the rate of change. In 1902, however, Henri asserted† that invert sugar retards the hydrolysis and ascribed the inhibiting effect almost entirely to the fructose.‡

In the same year, Adrian Brown§ also showed that invert sugar had a marked effect, whilst milk sugar had but little influence in retarding inversion.

Barendrecht has since published two communications|| dealing with the influence of a variety of substances. His results are of a most contradictory character and scarcely admit of rational interpretation; it is difficult to regard his radiation hypothesis seriously.

Apparently, the difficulties which attend experiments on the subject are not sufficiently appreciated. Invertase being extraordinarily active, it is necessary to work with highly dilute solutions of the enzyme: minute quantities of impurity therefore exercise an important influence. Unless hard glass vessels are used, it is impossible to obtain consistent results. A very large number of experiments have been made at various times (chiefly in 1904—05) to test the influence of glucose, fructose, galactose, mannose, dulcitol and mannitol: in many cases the results were conflicting, so that the problem cannot be regarded as solved: the general impression has been gained, however, that glucose and fructose alone retard the action, probably to an equal extent.

Conclusions similar to ours have been arrived at by Ford with reference to the action of diastase on starch,¶ and he has used amino-acids in order to neutralise impurities. From this point of view, we had used glycine and

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* 'Chem. Soc. Trans.,' vol. 57, p. 927.

† 'Compt. Rend.,' 1902, p. 917.

‡ This statement was incorporated in the table given in No. III.

§ 'Chem. Soc. Trans.,' vol. 81, p. 382.

|| 'Zeit. Phys. Chem.,' 1904, vol. 49, p. 456; 1906, vol. 54, p. 367.

¶ 'Soc. Chem. Ind.,' 1904, vol. 23, No. 8; 'Chem. Soc. Trans.,' 1904, vol. 85, p. 980. Ford expresses the opinion that the extraordinary results obtained with starch by various workers are due to a lack of recognition of the important influence of traces of impurity on the course of the change. The results of recent French workers justify this view. According to Ford, the action of diastase attains its maximum in neutral solution and is not augmented by asparagine unless there has been a previous restriction; this is true also of the various salts which are said to accelerate the action.

The accelerating influence of traces of acid on the activity of invertase (*cf.* O'Sullivan and Tompson) is probably a consequence of the neutralisation of alkalinity. We are inclined to think that the effects produced by salts are also of a secondary character and that they do not influence the action of invertase in any specific manner.

asparagine in experiments with maltase prior to the appearance of Ford's communication: finding them to be practically without influence, we felt safe in using them with invertase. As a rule, the activity of invertase is much increased by amino-acids; the experiments on which we rely were all made in presence of glycine.

Nature of Enzymes.—Granting that both glucose and fructose inhibit the action of invertase on cane sugar, it would seem to follow that this enzyme is so constituted that it can adapt itself to both sections of the biose. Unfortunately, no satisfactory expression of the structure of cane sugar has been arrived at hitherto, so that we are unable to formulate the changes which the biose undergoes on hydrolysis in a definite manner. It is an altogether peculiar substance, its extraordinary instability in presence of acids being most remarkable in comparison with that of other bioses* from which it does not differ markedly in most respects.

Invertase, the enzyme correlative with cane sugar, is equally remarkable among enzymes on account of its extreme activity.

Apparently, cane sugar is a derivative of α-glucose, to judge from O'Sullivan and Tompson's observations;† nevertheless it is not attacked by maltase and as this acts on all the *simple* α-glucosides, it cannot well belong to their class. Moreover, taking into account Pottevin's statement‡ that methyl-fructoside is not hydrolysed by extracts of *S. octosporus*, *Mucor mucedo* and *Mucor alternans*, which hydrolyse cane sugar and maltose, it must be supposed, in like manner, that cane sugar is not a *simple* fructoside.

These conclusions are in entire harmony with that derived from the study of inhibiting agents, so that there is little room for doubt that during hydrolysis the enzyme extends its influence over the whole of the cane sugar molecule—being intimately correlated in all its parts with the carbohydrate in the manner postulated in No. III§ of these communications.

The question arises whether this is true of other biases.|| The experimental material is not yet sufficient for the discussion of the problem, but there are already indications which make it probable—at all events, in the case of milk sugar—that what is true of cane sugar and its correlative enzyme applies generally.

It is commonly assumed that maltose is an α-glucoside, because maltase, which hydrolyses it, also acts on simple glucosides such as methyl-

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* *Cf.* IV, p. 530.

† *Cf.* E. F. Armstrong, 'Chem. Soc. Trans.,' 1903, vol. 88, p. 1308.

‡ 'An. Inst. Past.,' 3903, vol. 17, p. 43.

§ Pp. 522—524.

|| To avoid periphrasis, it appears desirable to term those enzymes which act on simple glucosides *monases* and those which attack the biose sugars *biases.*

α-glucoside. But independent proof of its character is afforded by the fact that the primary product of the hydrolysis of maltose is α-glucose (I.1307). Maltose and apparently lactose also appear to differ from cane sugar in that they are hydrolysed by enzymes which also act on the corresponding simple glucosides (monosides).

The existence of this difference affords further proof that the configuration of cane sugar and of its correlative enzyme are peculiar and altogether different from that of other bioses and biases. To account for the wider activity of a biase such as maltase, it is only necessary to suppose that not only are the simple glucosides of the same general type as the correlative bioses, but that this resemblance also extends to the biases and that it is sufficient in some cases if the attachment of the biase be secured over one section of the hydrolyte. Thus, representing the glucose radicle by G and other radicles such as are present in simple glucosides by R, E^g being the hypothetical radicle of the enzyme correlative with the radicle G, the following diagram may be used, the horizontal line representing the section of the molecule over which attachment of enzyme to hydrolyte takes place—

$$\frac{\mathrm{G\,.\,O\,.\,G}}{\mathrm{E}^g\,.\,\mathrm{E}^g}$$

$$\begin{array}{l}\underline{\mathrm{G\,.\,O}}\,.\,\mathrm{R}\\ \;\mathrm{E}^g\;\;.\;\mathrm{E}^g\end{array}$$

Formation of Enzymes.—It is conceivable that the enzymes themselves are subject to hydrolysis and simplification—in other words that a biase may give rise to a monase. The existence of monases in admixture with biases is, therefore, to be expected. Attention has already been called in the previous communication to evidence indicative of the presence in extracts of dried yeast of an enzyme (α-glucase) capable of hydrolysing methyl-α-glucoside, but without action on maltose; this discovery may well prove to be significant from the point of view now advanced.

There can be little doubt that the sucroclastic enzymes are products of hydrolytic changes conditioned by enzymes (mainly by proteoclasts, in all probability); the very different results obtained with extracts prepared under different conditions (at different temperatures, etc.) are scarcely to be accounted for in any other manner. And it is to be supposed that several enzymes may arise in this manner, all of one type, yet all capable of hydrolysing a particular hydrolyte and differing only in activity, much in the same way that the various simple glucosides represented by the general formula R′.O.G. (such as methyl-, ethyl-, phenyl-glucoside, salicin, etc.) differ in stability towards hydrolytic agents.

In the case of enzymes other than those which affect carbohydrates, the range of activity would appear, however, often to be greater than is ever manifest in the case of enzymes of the sucroclastic class. Thus lipase hydrolyses not only fats but also numerous ethereal salts; and many of the polypeptides prepared by Emil Fischer are hydrolysed by trypsin, which clearly exercises a wide range of activity.

As the only radicles common to the ethereal salts and to the polypeptides respectively are in the one case the group—CO.O—and in the other the group $>N.\dot{C}.CO$—present in the amino-acids represented generally by the formula $NH_2.CHR.CO.OH$, it is to be supposed that these simple groups are the active centres and that the specific influence of the enzyme is exercised at these centres. The variations observed in the activity of an enzyme towards a variety of hydrolytes are, from this point of view, to be ascribed mainly to differences in the stability of the hydrolytes; a single key, as it were, is provided which fits a variety of locks almost equally well; but as the locks are constructed with springs varying in stiffness, the effort which must be exerted to open the locks varies from lock to lock. But the interesting problem to be solved in this connection is whether, in the case of compounds such as the proteins, several junctions may not be resolved practically simultaneously by "compound" keys, as it were.

Bertrand's remarkable observations* on the limiting oxidising power of *Bacterium xylinum* have shown that configuration is of consequence even in the case of oxydases. It is conceivable that the oxidation of compounds such as the higher fatty acids is a regulated process consequent on the attachment of an oxidase to the terminal carboxyl group, the which oxidase serves to bridge over the interval between this group and some more or less distant CH_2 group and to locate oxygen against it.

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* 'Ann. Chim. Phys.,' 1904, ser. 8, vol. 3, pp. 181—288.

The Solubility of Air in Fats, and its Relation to Caisson Disease.

By H. M. Vernon, M.D., Fellow of Magdalen College, Oxford.

(Communicated by Dr. J. S. Haldane, F.R.S. Received April 1,—Read May 30, 1907.)

(From the Physiological Laboratory, Oxford.)

As is now well known, the very varied symptoms produced by rapid decompression from high atmospheric pressures, and popularly known as "caisson disease" or "diver's palsy," are due to liberation of bubbles of gas—chiefly nitrogen—in the blood and tissues. In the course of a recently published investigation, I found that air is much more soluble in certain oils than in water. Dr. J. S. Haldane pointed out to me the interest of this fact in connection with the causation of caisson disease, and at his suggestion I have repeated and extended my observations.

The fats experimented with were olive oil, cod liver oil and lard. In the case of the first two, the solubility was measured at 15° and at 37° C., whilst for lard it was determined at 45° C. In the observations made at room temperature, the oil was shaken violently with air in a bottle for several minutes, and was allowed to stand for 1 to $1\frac{1}{2}$ hours till all the air bubbles had risen to the surface. It was then weighed, and about 40 to 50 grammes of it were sucked up into the vacuous flask of a Geissler's mercury pump. This flask contained 70 to 100 c.c. of 0·5 per cent. sulphuric acid which had previously been well boiled for an hour so as to get rid of all traces of air. The mixture of oil and water was now boiled for half an hour, the oil breaking up into a very fine emulsion and giving up practically all of its gas in the first few minutes. This gas was pumped off and analysed with Haldane's* gas analysis apparatus. The oil was boiled with dilute acid instead of water, so as to obtain the whole of the carbon dioxide present, both combined and in solution. In determining the solubility at 37°, the oil, previously saturated at room temperature, was warmed to about 38° to 39°, and was shaken vigorously with air for about two minutes. At the end of this time its temperature had fallen to about 36°·5. It was warmed up a second time and the shaking repeated, and was then kept in a water bath at 37° for about half an hour in the case of the cod liver oil, and an hour in the case of the olive oil, these being the times required for all the bubbles of air to rise to the surface. The gaseous content of a weighed amount of the oil was then determined as before. The results obtained are given in the tables. They represent the volumes of gas, reduced to 0° and 760 mm., contained in 100 c.c. of the oil at the temperature recorded, when saturated with air at a pressure of 760 mm. To calculate these values, it was assumed that the specific gravity of olive oil at 15° is 0·917, and at 37°, 0·902.* The specific gravity of cod liver oil, compared with that of water at 15°, was found by direct experiment to be 0·928 at 15°, and 0·914 at 37°.

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* Haldane, 'Journ. Physiol.,' vol. 22, p. 465, 1898.

100 c.c. of olive oil contain—

	At 15° C.			Mean.	At 37° C.			Mean.
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Oxygen	2·20	2·23	2·42	2·28	2·33	2·36	2·30	2·33
Nitrogen	5·23	5·30	5·27	5·26	5·19	5·23	5·15	5·19
CO_2	0·19	0·24	0·16	0·20	0·17	0·13	0·18	0·16

100 c.c. of cod liver oil contain—

	At 15° C.			Mean.	At 37° C.			Mean.
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Oxygen	2·34	2·31	2·22	2·29	2·21	2·22	2·22	2·22
Nitrogen	4·95	5·15	5·07	5·06	5·05	5·10	5·08	5·08
CO_2	0·19	0·25	0·19	0·21	0·18	0·24	0·20	0·21

Considering only the mean values, we see that in the case of each oil the solubility at 37° is within the limits of experimental error the same as its solubility at 15° C. A similar constancy was found by Bunsen for the solubility of oxygen in alcohol at temperatures from 0° to 24°, though Timofjew† has since found that the solubility does slightly diminish with the temperature. In any case the diminution is small (6·8 per cent. diminution for oxygen and 5·2 per cent. diminution for nitrogen between 0° and 24°) and if it exists at all in the case of these oils, it must be smaller still. A simple proof of the slight effect of temperature on solubility is obtained by saturating the oil at room temperature, and heating it gradually in a water bath. Not a trace of gas is observed to come off if the temperature be kept at 97°, but at 100° it bubbles off in very small quantities.

The observations on lard were carried out at one temperature only. Lard melts at 36° to 48°, and solidifies at 27° to 30°, but if melted and shaken with air at 37° it is too viscid to allow the air bubbles to rise to the surface in a reasonable time. Hence the solubility was determined at 45°. The lard was heated to about 48°, and then shaken violently for a minute with air, its temperature at the end of this time having fallen slightly below 45°. The process was repeated, and after keeping the lard in a water bath at 45° for about 35 minutes the gases were boiled off in the usual way. They came off quite as readily as from the olive and cod liver oils. The data obtained are given in the table, they being calculated on the supposition that the specific gravity of lard at 45°, as compared with water at 15°, is 0·895.* If the mean values be compared with the mean values for the other fats, it will be seen that the solubility of oxygen is practically constant throughout. That of nitrogen varies slightly, it being 5·23 c.c. for olive oil (an average of the values at 15° and at 37°), 5·11 c.c. for lard, and 5·07 c.c. for cod liver oil. Still these differences are almost within the limits of experimental error. In the case of the lard, it is possible that the two minutes' shaking with air was not quite sufficient for complete saturation, but more thorough shaking was not possible, as it brought down a slight precipitate which prevented the rapid clearing of the air bubbles.

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* See Lewkowitsch, "Chemical Analysis of Oils, Fats and Waxes," London, 1898, p. 454.

† Timofjew, 'Zeit. Phys. Chem.,' vol. 6, p. 151.

	100 c.c. of lard at 45° C. contain—			Mean.
	c.c.	c.c.	c.c.	c.c.
Oxygen	2·23	2·40	2·35	2·33
Nitrogen	5·05	5·09	5·18	5·11
CO_2	0·12	0·12	0·15	0·13

In that fats of such very different composition as the three experimented with dissolve practically the same amounts of oxygen and nitrogen, we are fully justified in assuming that human fat would behave similarly. From the physical and chemical constants collected in the accompanying table,† we see that the melting point of human fat lies between those of cod liver oil and lard, whilst its iodine value is 61·5 per cent., or about the same as that of lard. In that cod liver oil has an iodine value of about 145 per cent., and

	Cod liver oil.	Olive oil.	Lard.	Human fat.
Solidifying point	0° to −10°	−6° (?)	27° to 30°	15°
Melting point ...	—	—	36° to 48°	17·5°
Specific gravity	0·928 at 15°/15°	0·917 at 15°/15°	0·895 at 45°/15°	0·903 at 25°/25°
Iodine value......	123 to 168 per cent.	79 to 88 per cent.	50 to 70 per cent.	61·5 per cent.

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* Lewkowitsch, *loc. cit.*, p. 564.

† Lewkowitsch, *loc. cit.*, pp. 454, 477, 562 and 564.

olive oil one of about 83 per cent., it follows that the proportion of unsaturated acids present in a fat has little or no influence upon its solvent powers for oxygen and nitrogen.

From the absorption coefficients for oxygen and nitrogen given by Bohr and Bock* it is calculated that 100 c.c. of water saturated with air at 15° absorb 0·733 c.c. of oxygen and 1·411 c.c. of nitrogen. Taking a mean of all the values obtained with cod liver oil and olive oil at 15°, the solubility of oxygen is found to be 3·1 times greater than in water, and of nitrogen 3·7 times greater. At 37° Bohr and Bock's values show that 100 c.c. of water absorb 0·507 c.c. of oxygen, and 0·975 c.c. of nitrogen. Taking a mean of all the values obtained with cod liver oil and olive oil at 37°, and with lard at 45° (for doubtless the solubility at this temperature is practically the same as at 37°), the solubility of oxygen is found to be 4·5 times greater than in water, and that of nitrogen 5·3 times greater. It has been found by Bohr,† that blood plasma dissolves 2·5 per cent. less nitrogen than an equal volume of water, whilst blood dissolves slightly less still; hence we may conclude that *at body temperature the fat of mammals dissolves at least five times as much nitrogen as water or as blood and blood plasma.*

The solubility of oxygen in fats, though apparently less than that of nitrogen, may in reality be as great. It is well known that oils are able to absorb and combine with oxygen, and the rate of this absorption is by no means inconsiderable. For instance, a sample of olive oil was saturated with air at 15°, and was kept for 23 hours at 18° in a stoppered bottle filled to the brim. It then yielded only 0·49 c.c. of oxygen per 100 c.c., whilst some of the sample from which the air was boiled off an hour after saturation yielded 2·40 c.c. Owing to its richness in unsaturated acids, cod liver oil absorbs oxygen more quickly still, and a sample saturated with air at 15°, and kept 23 hours at 18° in a stoppered bottle, gave only 0·05 c.c. of oxygen per 100 c.c. Undoubtedly, therefore, there must have been some absorption of dissolved oxygen during the half to one and a-half hours the air-saturated oils were kept before analysis.

It might be thought that the solubilities determined by the above described method are invalidated, or liable to be invalidated, by leakage of air into the vacuum pump. This is not the case if due precautions are taken. The pump must be in such good order that no appreciable leakage occurs when it is left vacuous for several days, and it must be kept vacuous for a week or more before use, for if air be left in it a small amount attaches itself to the glass walls of the apparatus, and only escapes slowly when the pump is made vacuous again. Air likewise attaches itself to the walls of the boiling flask, but it is got rid of by the preliminary boiling of the dilute acid previous to the introduction of the oil. In all analyses of gases, whether in oil, water, or salt solutions, such a preliminary boiling was invariably adopted, and by taking these precautions many hundreds of analyses have been made without invalidation of results by leakage of air. I have elsewhere* recorded nearly a hundred analyses of the gases in sea water, and the mean nitrogen values then obtained are almost identical with the nitrogen absorption values determined by Dittmar.

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* Bohr and Bock, 'Wied. Ann.,' vol. 14, p. 318, 1891.

† Bohr, 'Nagel's Handb. d. Physiol.,' vol. 1, p. 62, 1905.

The bearing of the above recorded results upon many phases of caisson disease is probably a direct one. The commonest symptoms observed in caissoniers consist of joint and muscular pains, and in paralysis, the latter being the result of injury to the spinal cord. Thus in autopsies of caissoniers, Bert observed softening of some inches of the spinal cord in the dorsal region. v. Leyden, in a case of death 15 days after decompression, observed signs of myelitis, and small irregular fissures in the mid-dorsal cord, probably produced by the escape of gas. Autopsies of divers have shown necrobiosis and hæmorrhages in the spinal cord,† and distention of the blood vessels by air bubbles.‡

Chemical analysis shows that spinal cord and peripheral nerves contain nearly 20 per cent. of fat and fat-like substances. Adipose tissue contains about 83 per cent. of fat, and yellow bone marrow (as distinct from red marrow, which is poor in fat) contains no less than 96 per cent. of fat (Gorup-Besanez). If these tissues were saturated with nitrogen at, for instance, four atmospheres of atmospheric pressure, 100 c.c. of them would contain, instead of the $0{\cdot}975 \times 4 = 3{\cdot}90$ c.c. of dissolved nitrogen present in an equal volume of water, about 7 c.c. in the case of the spinal cord and nerve, and 20 c.c. in the case of yellow bone marrow. On sudden decompression, therefore, the volume of nitrogen bubbling off in a gaseous form might be two to six times as much as that from the non-fatty fluids and tissues of the body.

It has been found that the severity of the symptoms developed by caissoniers is influenced greatly by the time of exposure to increased pressure. In sinking the foundations of a bridge at St. Louis, a pressure reaching at a maximum to 50 lbs. above normal was used, and a large proportion of the workers were affected. As the depth of the caissons increased, the shifts were shortened from four hours to one hour, with the result that the serious accidents were reduced to *nil.*[*] Presumably, therefore, the body tissues were not so fully saturated with nitrogen in the shorter as in the longer period. On the other hand, Hill and Greenwood[†] found that on exposing themselves to a pressure of 30 to 45 lbs., their urine became saturated with the theoretical amount of nitrogen in 10 to 15 minutes. This quicker saturation must be due to the blood-flow through the kidney being much more rapid than that through the spinal cord, and very much more rapid than that through adipose tissues. Even if it were as rapid through fat-containing tissues as through the kidney, the fats, in virtue of their greater solvent power, would take longer to become fully saturated than the fluids of non-fatty tissues.

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* Vernon, 'Journ. Physiol.,' vol. 19, p. 68, 1895.

† Cited by Hill and Macleod, 'Journ. Hygiene,' vol. 3, pp. 408 and 409, 1903.

‡ See Hill's "Recent Advances in Physiology," p. 250, 1906.

Conclusion.

At body temperature, fats dissolve more than five times as much nitrogen as an equal volume of water or blood plasma.

The special tendency of the fat-containing tissues (such as subcutaneous tissues, spinal cord and peripheral nerves) of caisson workers and divers to suffer injury from the liberation of gas bubbles after rapid decompression is dependent on this great solubility.

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[*] See Paul Bert's "La Pression Barométrique," Paris, 1878, p. 404.

[†] Hill and Greenwood, 'Roy. Soc. Proc.,' B, vol. 79, p. 21, 1907.

On Mitosis in Proliferating Epithelium.

By J. O. Wakelin Barratt, M.D., D.Sc. Lond.

(Communicated by Professor C. S. Sherrington, F.R.S. Received April 4,—
Read May 30, 1907.)

(From the Cytological Laboratories, University of Liverpool.)

The object of the present investigation is to determine the character of the mitosis occurring in cutaneous epithelium which has been artificially stimulated to proliferate freely. In the epithelial proliferation of carcinoma, in addition to somatic mitosis, a synaptic mitosis is also met with.* In attempting to decide upon the significance to be attached to this latter phenomenon it is necessary to ascertain whether reduction mitosis is met with exclusively in cancerous proliferation of the surface epithelium, or whether it is also encountered in non-cancerous proliferation. If it is present in the latter case, then synaptic mitosis cannot be regarded as exclusively related to epithelioma, or, for that matter, to sexual reproductive tissue; if, however, only somatic mitoses occur in non-cancerous epithelial proliferation, then the possibility is present that the altered biological characters presented by the epithelial cells of malignant growths may be in some way directly connected with the reduction taking place in the number of chromosomes.

The abundant epithelial proliferation occurring in many cutaneous diseases, *e.g.*, psoriasis, lichen planus, and dry forms of eczema, is unsuitable for this investigation, since the amount of material available from such sources is too small and the supply too inconstant to fulfil the requirements of research. Recourse was therefore had to epithelium, which had been made to proliferate freely by the use of scharlach R,† dissolved in olive oil, the injection of which beneath the skin of the rabbit's ear causes extreme and, it may, irregular hypertrophy of the Malpighian layer.‡ It was further found of advantage in obtaining an abundance of division figures to implant pieces of such proliferating epithelium under the skin of the rabbit. Using the material obtained in these two ways the present investigation was carried out. The effect of scharlach R, it may be observed, is to cause marked increase of the prickle layer, both of the surface epithelium and of the hair follicles. This increase is at first fairly regular, but later, when considerable in degree, becomes irregular, and ingrowth of epithelium occurs, comparable to, though considerably less extensive than, that occurring in squamous epithelioma. If the proliferation be set up in scar tissue, the resemblance to the latter condition is striking. With the disappearance of the scharlach R the process comes to an end and involution occurs, though the skin appendages may be more or less destroyed. The character of the epithelial proliferation is, therefore, intermediate between that of such affections as psoriasis and lichen planus, in which epithelial hypertrophy without downgrowth takes place, and epithelioma, in which downgrowth of epithelium is the characteristic feature, and is the means by which neighbouring tissues are invaded and extension of the growth occurs.

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* Farmer, Moore, and Walker, 'Roy. Soc. Proc.,' B, vol. 77, p. 226.

† Scharlach R is azo-orthotoluol-azo-β-naphthol : $C_7H_7N = NC_7H_6N = NC_{10}H_6OH$.

‡ B. Fischer, 'Münch. Med. Wochenschr.,' 1906, 53. Jahrg., S. 2041.

When marked proliferation of the epithelium of the rabbit's ear has been brought about under the conditions above described, sections hardened in Flemming's solution and stained by Heidenhain's iron alum hæmatoxylin method show numerous mitoses, amounting occasionally to as many as five mitoses to every hundred cells encountered. The mitotic cells are of large size, being 10 μ to 16 μ in diameter. In most cases the individual chromosomes can be recognised to be more or less curved rods of varying length, too closely-packed to be counted. In a relatively very small number of the mitotic figures, however, it is possible to enumerate the chromosomes, and in this way to determine the forms of mitosis present.

The form most commonly met with appears to be the ordinary somatic type shown in fig. 1,* which presents no essential points of difference from the corresponding type in the testis of the rabbit, taken as a standard,

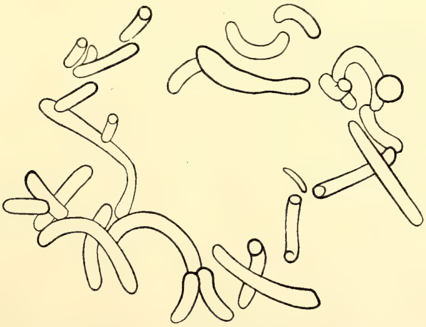

Fig. 1.—Normal Somatic Mitosis in Proliferating Epithelium of Rabbit's Ear.

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* The mitoses exhibited in this and the succeeding figures, all of which have the same magnification (*cp.* scale in fig. 5), were drawn to scale in plan and elevation, and were then modelled. The illustrations were prepared from the models. The use of models was adopted in order to permit a simultaneous view of all the chromosomes present, in their natural relation, instead of the succession of views at different levels afforded by the microscope.

shown in fig. 2. In this form the number of the individual chromosomes which could be distinguished ranged between 28 and 32, while in the testis, taken for comparison, the number of chromosomes which could be recognised lay between 28 and 36. In the somatic mitoses of the ear of the rabbit, after injection of scharlach R, the chromosomes appear larger and coarser than in the testis, but are otherwise of the same type.

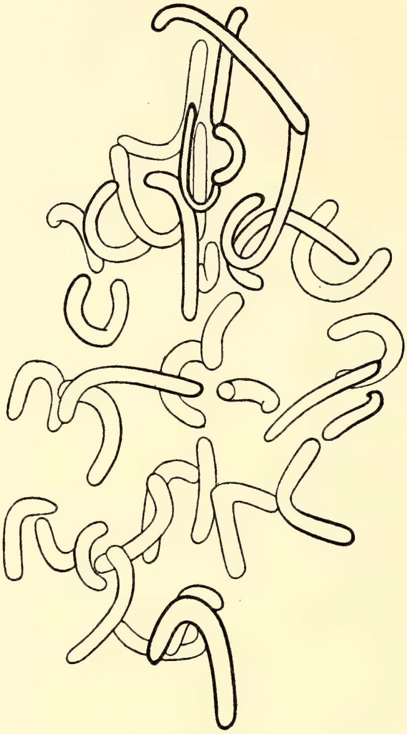

Fig. 2.—Normal Somatic Mitosis in Testis of Rabbit.

In addition to the above, another type of mitosis, shown in fig. 3, is met with less frequently than in the former. Here the chromosomes, instead of being curved rods, mostly assume the form of large spheroidal or ellipsoidal masses, taking the stain chiefly in their peripheral layer and to a less extent centrally; some of the chromosomes, however, form short rods, straight or curved, occasionally swollen at the extremities; again, some of the chromosomes form long rods (two such are shown in fig. 3, one thick, one thin). All these chromosomes are arranged in pairs, with or without connecting threads of chromatin, in the same manner as the so-called gemini of the first maiotic division of the testis (fig. 4), with the exception of the two last mentioned, which are evidently equivalent to pairs, though no division or constriction can be recognised. It will be seen that the chromosomes in figs. 3 and 4 are on the equator of the spindle. In fig. 3 the number of pairs is 14; in other mitotic forms of the same type it was found to vary from 14 to 18. A glance at the heterotype mitosis from the testis of the rabbit (fig. 4), in which the number of gemini or pairs of chromosomes which could be counted was found to vary from 14 to 18, shows the essential morphological identity of the synaptic form occurring in the proliferating epithelium of the rabbit's ear (fig. 3) with the first maiotic mitosis in the testis of the same animal (fig. 4). In both forms the

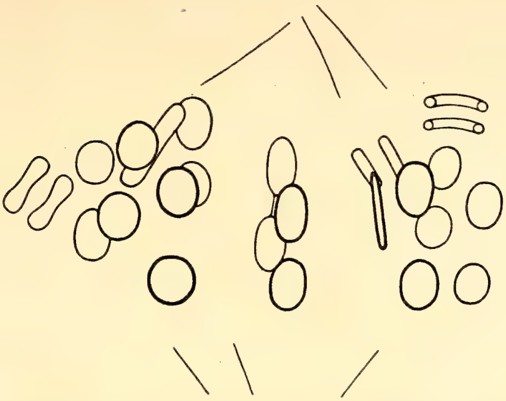

Fig. 3.—Reduction Mitosis in Proliferating Epithelium of Rabbit's Ear.

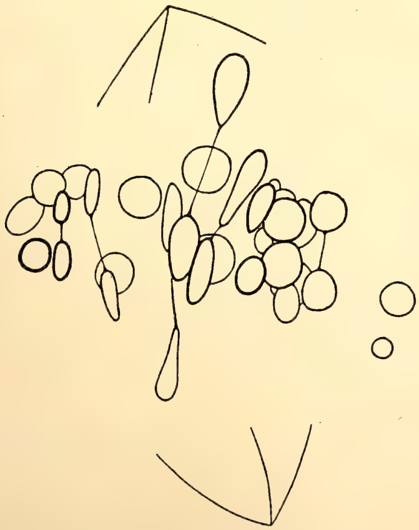

Fig. 4.—Heterotype Mitosis in Testis of Rabbit.

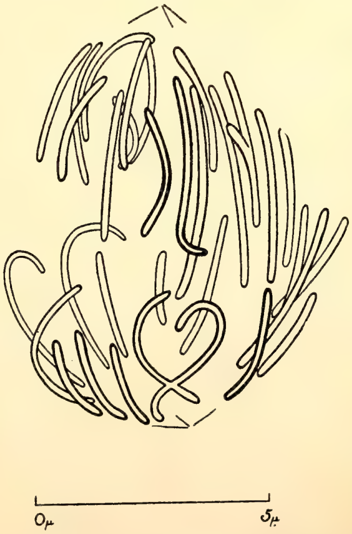

Fig. 5.—Post-synaptic Mitosis in Proliferating Epithelium of Rabbit's Ear.

chromosomes are for the most part large spheroidal or ellipsoidal masses, minor differences being recognisable in the smaller gemini.

In addition to the above type, another less frequent form of mitosis was encountered. This is exhibited in the dyaster shown in fig. 5. Here the chromosomes, which form elongated narrow rods, curved or bent, are also reduced in number, varying from 16 to 18 in each aster. This form may represent a post-synaptic division. If this is so, it is impossible to determine whether it is the first or a subsequent post-synaptic division, since there is no means of identifying its particular generation.

Spireme forms are fairly common. In these the chromatin granules are not unfrequently abundant, but well-defined double beading of the spireme thread has not so far been observed. It may be noted, however, that in the testis of the rabbit double beading could not be clearly exhibited. Late prophases, in which different permanent forms of the chromosomes composing the gemini, similar to those described by Moore and Arnold,* were recognisable, have not been met with, nor have masses of archoplasm or archoplasmic vesicles been up to the present encountered in proliferating epithelium of the rabbit's ear.

No suggestion can be offered, from the observations made in the course of this investigation, as to the significance of the reduction division which takes place. Nor has it been possible to ascertain the further history of the reduced cells, and, in particular, to determine whether division with the reduced number of chromosomes may be repeated for more than one generation.

It may be observed that the number of chromosomes counted has not been constant, but has varied from 28 to 36 in somatic mitoses, and from 14 to 18 in the reduction mitoses. The difficulty of accurately ascertaining the number present under the conditions obtaining in stained sections viewed under high magnification may in part account for the variation met with, particularly in the former case. It is, however, not at all clear that this explanation is completely satisfactory; on the contrary, the constancy with which variation is met with suggests, particularly when reduction occurs, and enumeration of chromosomes becomes in consequence much less difficult, that the number is not absolutely fixed, but exhibits a certain degree of variation.

Considerable variation was exhibited in different experiments in respect of the number of division figures encountered. As a rule, the number was greatest when the epithelial proliferation was most marked. An extreme degree of mitosis was observed at the end of a week in some of the implanted portions of skin. The reduction mitoses were met with in those cases in which proliferation was considerable, but owing to the very large number of division figures which are not sufficiently well defined to enable their character to be determined, it is not possible to give statistics of the relative frequency of the somatic and reduction mitoses occurring at different periods in the proliferating epithelium.

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* 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 563; also Moore and Embleton, *ibid.*, p. 555.

Summary.

The preceding observations may be briefly summarised as follows :—

1. In epithelial proliferation brought about by scharlach R, both normal somatic and reduced mitoses occur. This statement applies to epithelium proliferating *in situ,* and also to the same implanted under the skin.

2. In the reduction mitoses the number of chromosomes which could be counted varied from 14 to 18. In the somatic form the number counted varied from 28 to 36.

3. Reduction mitoses could be recognised less frequently than somatic mitoses.

4. Post-reduction mitoses were met with.

5. The character of the mitoses occurring was not definitely altered by implantation under the skin.

Increase in the Complement-content of Fresh Blood-serum.

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(Communicated by Professor Gotch, F.R.S. Received March 26,—Read May 9, 1907.)

Very soon after coagulation has occurred in blood withdrawn from the vessels, serum begins to be expressed. The amount, at first small, increases rapidly, and in a few hours, at room temperature, contraction of the clot is practically complete. Some observations made in the course of other work suggested that the composition of the serum first yielded differs from that of the serum expressed later, and a special investigation of the point led to the results recorded in this paper. The serum given off from the clot at varying intervals of time after bleeding was tested for its content in hæmolytic complement. This body was selected for examination, not only because it can conveniently be studied in the test-tube, but also because much greater quantitative accuracy of result can be obtained with it than with, for example, a bactericidal complement. In most of the experiments, the serum of rabbits was tested against the red blood-corpuscles of the ox, and the necessary immune-body was derived from the serum of rabbits immunised by repeated injections of these corpuscles. In some instances other combinations were used, as is stated later, but in every case the red cells used for injection and for examination were fresh, and had been freed from serum by being washed thrice in many times their bulk of isotomic salt solution. The experiments fall into two groups, according as the serum examined was from a normal animal or from an animal that had been previously immunised.

1. *Normal Serum.*

The method adopted was as follows:—A rabbit was treated with injections of ox red blood-corpuscles, and when its serum had reached a sufficiently high titre, its blood was withdrawn and the serum obtained. This was then heated for half an hour at 56° C. to destroy the contained complement, and served as a stock of "immune-body" (by which name I shall refer to it in the rest of this paper). The neck of a normal rabbit having been shaved and sterilised, the large vessels of the neck were cut, and the issuing blood was caught in a sterile flask. As soon as coagulation had occurred, the clot was loosened from the sides of the flask by a sterile glass-rod. At varying intervals of time, samples of the separating serum were taken and rapidly centrifuged to free them from cells, and the supernatant fluid was then tested. Each sample was examined separately as soon as it was obtained, instead of waiting for the last sample and examining them all together. The latter method was used as a control in a few cases, but the interval between the taking of the first and last samples extended over several hours, during which time spontaneous deterioration or other change of the complement might easily occur in the earlier samples. Such a change does, in fact, occur, as will be shown later, and it was necessary to avoid this source of error.

The examination of a sample was made in the following way:—A series of test-tubes was prepared, and into each was measured the same amount of immune-body (the necessary dose having been previously ascertained). Diminishing amounts of the sample of serum were then added to the test-tubes, the first tube receiving a large dose, the next a slightly smaller, the third a still smaller, and so on. The volume of fluid in all the tubes was then made the same by adding salt solution, and finally 4 c.c. of a 1-per-cent. suspension in salt solution of ox corpuscles were added to every tube. Control-tubes having been prepared, all tubes were shaken and placed in an Ostwald water-bath at 37° C. After two hours the tubes were withdrawn, and of the series the lowest tube, in which total hæmolysis had occurred, was noted. The dose of serum in this tube contained just enough complement to produce total hæmolysis in that time. The same procedure was carried out with every sample, the amounts of immune-body and red cells being kept constant throughout. An example of such an experiment is given below.

Example 1.—Dose of immune-body = 0·04 c.c.; 4 c.c. 1-per-cent. suspension ox red blood-corpuscles. Total = complete hæmolysis. Time, two hours at 37°.

Dose of serum in c.c.	Time in hours between bleeding of rabbit and withdrawal of sample.						
	$\frac{1}{3}$.	$\frac{1}{2}$.	1.	2.	$3\frac{1}{2}$.	5.	7.
0·4 ……	total	total	total	total	total	total	total
0·35 ……	,,	,,	,,	,,	,,	,,	,,
0·3 ……	,,	,,	,,	,,	,,	,,	,,
0·25 ……	not total	,,	,,	,,	,,	,,	,,
0·2 ……	less	not total	,,	,,	,,	,,	,,
0·15 ……	…	less	not total	,,	,,	,,	,,
0·1 ……	…	…	less	not total	,,	,,	,,
0·08 ……	…	…	…	less	not total	,,	not total
0·06 ……	…	…	…	…	less	not total	less
0·05 ……	…	…	…	…	…	less	

No lysis was produced by 0·35 c.c. of the 5-hours sample without immune-body; nor by 0·04 c.c. of the immune-body without the normal serum.

In this case, then, 0·3 c.c. of the serum, withdrawn 20 minutes after bleeding, was required to produce total lysis; but with the successive samples the amount required steadily decreased, until of the serum which had remained five hours in contact with the clot 0·08 c.c. was sufficient. The amount of complement contained in the later sample, or at least its activity, must be more than three times as great as that of the complement in the earliest sample. It increased steadily for five hours, and then began to diminish (decreasing afterwards till it eventually disappeared).

This example is typical. In all the experiments (seven) done with normal rabbit's serum and ox corpuscles, a similar increase in the complement has been observed. The amount of the increase varies, but as a rule it is to double, sometimes to three times, the quantity found in the first sample. The time during which increase occurs also varies. The longest time observed was 14 hours; but this is exceptional, and in most cases the rise is complete in five to seven hours. The same phenomenon occurs when, instead of ox corpuscles and the appropriate immune-body, horse corpuscles are used with immune-body from rabbits treated with injections of horse corpuscles. When normal guinea-pig serum is used instead of rabbit serum to supply the complement for either ox or horse corpuscles (with appropriate immune-body from rabbit), the same increase to about double the amount is found. The duration of the increase, however, is shorter, extending to two to four hours. (In one of six experiments with guinea-pig serum the rise was very slight, and complete in one and a-half hours.)

It may be suggested that this increase in the complementing-power is only apparently a change in the serum, and that the change really occurs in the red corpuscles used as the reagent. In the interval between the examination of the first and last samples the red cells may become more sensitive, and so give a false appearance of increased power in the serum. This, however, cannot be the explanation of the facts observed. The red corpuscles were kept in the cold room (about 3° C.) till immediately before use, and were always fresh. Under these conditions they do not become perceptibly more sensitive within so short a time as seven to eight hours, when tested with other lytic bodies, such as bacterial hæmolysins and chemical reagents. Further, the results, described later, obtained with immune sera prove that no such change as is here suggested occurs in the corpuscles used.

It was of interest to determine whether this change in the complement-content of serum occurs even when the serum is removed from the clot, or takes place only when it remains in contact with the clot. This was investigated in a number of the normal serum experiments. A portion of the first sample was set aside in the cold after centrifuging, and examined some hours later, at the same time as a sample which had remained on the clot during the same period. The results show that a rise does occur in the serum, even when separated from the clot. The amount of this rise is, however, very small, only a small fraction of the increase which has taken place in the serum left on the clot. *E.g.*, in the example given above, the value of the 20 minutes sample rose in five hours, when off the clot, from 0·3 c.c. only to 0·2 c.c., as against the rise to 0·08 c.c. of the serum left on the clot during that time. It is not easy to suggest an explanation of this phenomenon. We might suppose that complement exists in serum or plasma partially in an inactive or zymogen phase, which is slowly converted into the active body. Or again, on the view that complement is derived from leucocytes, we may suggest that even after prolonged centrifugalisation, fragments of white cells remain suspended in the serum and gradually disintegrate. Such explanations, however, have as yet no real experimental basis, and we know too little of the nature and source of complement to make speculations profitable.

In the normal sera examined, then, hæmolytic complement increases by 100 to 200 per cent. during the first few hours after the drawing of blood, the duration of the increase extending in the case of the rabbit over a period of five to seven hours, in the case of the guinea-pig over a period of two to four hours. It is probable that this variation is not peculiar to the hæmolytic complements. Ainley Walker (1) found the bactericidal action of serum to increase during the first few hours after the blood was shed, and attributed this to an increase in the amount of complement. According to Macdonald (2), whose results have been confirmed by Craw, the opsonic power of serum undergoes a similar increase.

J. H. Craw has pointed out that all these bodies are relatively unstable, while the hæmolytic immune-body, which does not vary, is comparatively stable; but whether this fact is of significance can only be determined when further evidence has accumulated on the subject. The results obtained in the experiments recorded here have no immediate bearing on Metchnikoff's view that alexine does not exist as such in circulating plasma. Sufficient serum for examination does not separate until 15 to 20 minutes have elapsed from the time of bleeding. In the serum then obtained the amount of complement, though much less than is found later, is still always considerable, and there was nothing to indicate that at the moment of bleeding the blood contained no active complement.

Example 2.—Dose of immune-body: 0·025. Horse red blood-corpuscles: 4 c.c. of 1-per-cent. suspension; two hours at 37°. Quantity of normal rabbit serum necessary for total hæmolysis was, of 1 hour sample, 0·6 c.c. $2\frac{1}{2}$ hours sample, 0·5 c.c.; 5 hours sample, 0·3 c.c.; $7\frac{1}{4}$ hours sample, 0·325; 9 hours sample, 0·35 c.c.; 12 hours sample, 0·4 c.c. Hence a rise to double the amount in 5 hours. In the control tubes, 0·5 c.c. of the 1 hour sample gave no lysis, but the same dose of the 5 hours sample gave a trace.

Example 3.—Dose of immune-body, 0·04 c.c. Ox corpuscles: 4 c.c. 1-per-cent. suspension; 2 hours at 37°. Quantity of normal guinea-pig serum necessary for total hæmolysis, of $\frac{1}{2}$ hour sample, 0·05 c.c.; of 1 hour sample, 0·025 c.c.; of 4 hours sample 0·03 c.c.

2. *Immune Serum.*

The immune sera examined were those of animals, mostly rabbits, injected with blood-corpuscles, usually of the ox. In some cases the serum came from rabbits, which had been injected with horse corpuscles, and in one case from a goat injected with ox corpuscles. The results in all the cases were concordant. Two factors in such sera require to be examined: the immune-body and the complement.

The immune-body is easily tested, since it does not appreciably, if at all, deteriorate spontaneously. The immunised animal was bled as before, and samples of serum withdrawn in the usual way. Each sample, after centrifuging, was at once heated 30 minutes at 56° to destroy the complement, and, when the last was ready, all were examined together. The method was similar to that described in the last section. With a constant quantity of corpuscles and a constant quantity of serum from a normal animal as complement, the minimum dose of each sample necessary to produce total lysis was determined. This was found to be the same for all the samples; and in all the experiments this proved to be the case. The immune-body, therefore, does not vary, but is present in its full amount from the first.

Example 4.—Dose of normal rabbit serum as complement, 0·15 c.c.; 4 c.c. 1-per-cent. suspension ox corpuscles; 2 hours at 37° C. Immune serum from rabbit injected with ox corpuscles. The dose required of this serum after heating was for the 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and 12 hours samples the same, viz., 0·04 c.c. The dose of the 10 hours sample was 0·045 c.c., which is probably an experimental error.

The investigation of the complement in immune serum is more difficult. We have no means of destroying immune-body, and leaving complement in the serum, and Ehrlich's (3) method of absorption of the immune-body in the cold is not suited to quantitative estimations of this kind, and has been shown by Mainwaring (4) to be open to serious objections. On the other hand, we cannot simply ignore the immune-body contained in the serum. By taking varying doses of the serum we should be taking not only varying quantities of complement, but also varying quantities of immune-body; and Morgenroth and Sachs (5) have shown that the amount of complement necessary for hæmolysis depends in some measure on the amount of immune-body present. Now, in the process of immunising, the animal responds by a great increase of immune-body in its serum, but there is no corresponding increase of complement. Of the serum of a highly immunised animal, therefore, it is possible to find a dose which contains enough immune-body to cause total laking of the chosen quantity of corpuscles, but does not contain enough complement. Complete hæmolysis, therefore, does not occur with that dose, unless we make good the lack of complement by adding some from another source. By making use of this fact, a method was devised of estimating the complement in immune serum. An immunised animal was bled and samples of serum were withdrawn from the clot in the usual way, each sample being tested at once after centrifuging. Such a dose as has been just described, containing enough immune-body but not enough complement, was taken, and the same dose was taken for all the samples. Since, as we have just seen, the immune-body does not vary in the different samples, we are thus taking always the same amount of immune-body. The deficiency in complement was supplied by adding the serum of a normal rabbit, and the quantity of this serum which had to be added was carefully determined for each sample. The greater the quantity of normal serum required, the less complement must the dose of immune serum contain. The amount of normal serum that would be required, if the dose of immune serum contained no complement, is readily found by first heating the latter and so destroying its contained complement. The difference between this amount and the amount we had to add when the sample was unheated gives us a measure of the value of the complement contained in the unheated dose, this value being expressed in terms of the normal serum. We can thus determine the amount of complement contained in each sample, and so ascertain the extent of the variation, if any.

Example 5.—Four cubic centimetres of 1-per-cent. suspension of ox corpuscles; 2 hours at 37° C. Immune serum from rabbit, injected with ox corpuscles; of this serum, when heated, 0·02 c.c. required the addition of 0·25 c.c. normal rabbit serum (*i.e.*, complement) to produce total hæmolysis, and this quantity of the normal serum by itself produced no lysis.

c.c.				c.c.	
To 0·02 unheated immune serum,	⅓	hr. after bleeding,	must be added	0·225	normal serum.
„ „	1	„	„	0·2	„
„ „	2	„	„	0·225	„
„ „	3¾	„	„	0·25	„
„ „	5	„	„	0·25	„

0·02 c.c. unheated serum by itself, no perceptible lysis.

Since 0·25 c.c. normal serum was required, when the immune serum had been heated, there was contained in the dose of the unheated immune serum—

				c.c. c.c.	c.c.	
Of the	⅓	hr. sample complement	equivalent to	0·25—0·225 =	0·025	of the normal serum.
„	1	„	„	„	0·05	„ „
„	2	„	„	„	0·025	„ „
„	3¾	„	„	„	No appreciable amount.	
„	5	„	„	„	„ „	

The amount of complement increased to double the amount by the end of the first hour, but had decreased again after two hours, and by four hours had become inappreciable.

Example 6.—Immune serum from rabbit injected with ox corpuscles; of this when heated 0·05 c.c. required 0·13 c.c. normal rabbit serum. To 0·05 of the immune serum unheated—

				c.c.	
From the	⅓	hr. sample	must be added	0·1	normal serum.
„	1	„	„	0·085	„
„	1½	„	„	0·08	„
„	3	„	„	0·1	„
„	5	„	„	0·1	„

				c.c.	
Therefore the dose of the	⅓	hr. serum	contained the equivalent of	0·03	normal serum.
„ „	1	„	„	0·045	„
„ „	1½	„	„	0·05	„
„ „	3	„	„	0·03	„
„ „	5	„	„	0·03	„

The complement increased by about 66 per cent. in 1½ hours.

By this method we can demonstrate the occurrence of change in the quantity of complement contained in the immune serum, and can determine the time during which such change is occurring. It is true that we supplement an "immune" complement by a "normal" complement; and it is probable that partial complements do not exist in the same proportions in the two sera. It is even possible that they are not identical, even when derived from animals of the same species, although there is no evidence of this, and such evidence as we have is against this view. Even if it were so, however, the fact would not invalidate the method, in so far as it indicates the occurrence of change and the duration of that change. The amount of change which it shows to occur is, however, more open to question. It is assumed that the immune-body undergoes no alteration in the process of heating, and this, probable as it may be, has not been proved. Further, the existence of different proportions of partial complements in the two sera would affect the quantitative accuracy of the results obtained. In any case, great stress should not be laid on particular figures, since the experimental error is quite appreciable, though remarkable regularity and accuracy are obtainable with hæmolytic sera. While we may rely, then, on the method showing us whether any change occurs, and the duration of such change if present, the amount of change indicated is probably only an approximate measure of what really occurs.

In example No. 5 given above, the amount of complement had risen by the end of one hour to double the amount first observed. As a rule, the increase is rather less. In some of the experiments (10 were made with immune sera) no increase at all was found, the complement diminishing steadily from the first observation. Further, in all the experiments where any rise took place it was complete in one and a-half to two hours after the blood was drawn.

There is a difference, therefore, between normal and immune serum in this respect. In normal serum we found the complement to increase by 100 to 200 per cent. and the duration of the increase to be from five to seven hours. In immune serum the full amount of complement is often present from the first, and where an increase does occur, it is complete in a short time and is not so great as the increase in normal serum. By the process of immunising, therefore, complement, or the source of complement, has been altered in such a way that it is developed in the serum with greater readiness than is the case with normal blood. The repeated injections of red cells may have induced repeated new formations of complement, with the result that the leucocytes or other cells have been "educated," in Metchnikoff's sense, to secrete complement, or its precursor, more quickly than where such stimuli have not been given. But here, again, explanation becomes pure hypothesis.

On the suggestion of Professor Muir, an experiment was made to see whether the change produced by the process of immunising affected all the hæmolytic complements of the animal immunised or only those specially concerned in the laking of the red cells used for the immunisation. A rabbit immunised against ox corpuscles was bled, and samples of serum withdrawn in the usual way. Two series of experiments were then carried out. In the first, ox corpuscles and the appropriate immune-body were used, and the variation of the complement determined by the method for immune serum.

The rise was complete in one and a-half hours, and amounted to under 70 per cent. In the second series, guinea-pig corpuscles were used along with immune-body derived from a rabbit immunised to these cells. Here the samples were treated as if they were samples of a normal serum, and the minimal dose giving total hæmolysis with constant amounts of immune-body and corpuscles was determined for each. The rise of complement was not complete after three hours, and amounted to more than 100 per cent., *i.e.*, it followed the rule of variation of a normal serum. The change produced by immunising with ox corpuscles in this case, therefore, did not affect all the complements laking guinea-pig corpuscles, but was confined to the ox-cell complements. As only one such experiment was made, it is impossible to say whether this is a general rule or not, or in what degree the complements, which are able to take part in lysis of either ox or guinea-pig corpuscles, are affected.

Summary.

1. The serum first separating from shed blood may differ in composition from that separating later.
2. With normal blood of rabbit or guinea-pig, the amount of hæmolytic complement contained in the first serum to separate is comparatively small, and progressively increases in serum separating later for a period of 5 to 7 hours in the case of rabbit, 3 to 4 hours in the case of guinea-pig, to reach a maximum 2 to 3 times as large as the amount first observed.
3. Even after removal from the clot, the amount of complement may increase in serum which separated soon after the blood was drawn, but this increase is slight.
4. This increased activity of serum is not peculiar to the hæmolytic complements.
5. With the blood of immunised animals, immune-body does not increase in this manner.
6. With the blood of immunised animals, the hæmolytic complements may increase in amount, but this increase is not always present, and when it does occur is less, and reaches its maximum more quickly than is the case with normal blood.
7. The alteration thus produced by the process of immunising probably affects only the hæmolytic complements related to the immunising agent, and not other hæmolytic complements which may be present in the serum.

A portion of this work was done at Queensbury Lodge, Aldenham, and I desire to express my thanks to the Directors of the Lister Institute of Preventive Medicine, and also to Dr. Dean for the facilities afforded me

there. I am glad also to have this opportunity of thanking Professor Ritchie, of Oxford, for the interest he has taken in my work and for suggestions made in the course of it.

REFERENCES.

1. Ainley Walker, "On some Factors in Bacteriolytic Action," 'Journal of Hygiene, vol. 3, p. 52, Jan., 1903.
2. Macdonald, G. G., "On Immunity in Pneumococcal Infections," in 'Studies in Pathology,' edit. Bulloch, Aberdeen, 1906.
3. Ehrlich and Morgenroth, 'Berlin Klin. Wochenschr.,' 1899, No. 1.
4. Mainwaring, 'Journal of Infectious Diseases,' vol. 2, pp. 446 and 460.
5. Morgenroth and Sachs, 'Berlin Klin. Wochenschr.,' 1902, No. 5.

The Correlation of the Ovarian and Uterine Functions.

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(Communicated by Professor E. A. Schäfer, F.R.S. Received April 10,—Read May 30, 1907.)

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A considerable body of evidence has been adduced in support of the hypothesis that the uterus is dependent upon ovarian influence for the maintenance of its structure and the discharge of its functions, and that the removal of this influence at any time during reproductive life causes the uterus to atrophy, besides producing a profound effect upon the entire metabolism of the organism. Some writers, on the other hand (*e.g.*, Blair Bell), contend that it is the uterus which is of prime importance, and observations have recently been cited pointing to the conclusion that this organ exercises a control over the growth and activity of the ovaries. Others, again (*e.g.*, Bond), adopt the view that the ovaries and uterus are to a very large extent inter-dependent upon each other.

The clinical evidence brought forward by surgeons after removal of the uterus and ovaries in the human subject has supported to a great extent the experimental evidence of the existence of such an inter-relation. The modern tendency to conserve these organs as far as possible during surgical interference has been brought about largely by the belief in the dependence of the one organ upon the other. Important work bearing upon this subject has been published recently by Zweifel (1899), Mandl and Bürger (1904), Bond (1906), and many other surgeons.

It is the purpose of this paper to record a series of experiments dealing with these questions, and to state the conclusions which we have reached regarding them.

Influence of the Ovary upon the Uterus.

A brief *résumé* of the experimental evidence adduced upon the existence and nature of the ovarian influence has been recently given in a paper by Marshall and Jolly (1907), who cite observations showing that the uterus undergoes undoubted degenerative changes after the removal of the ovaries. Further evidence, which is in accord with this conclusion, has been independently reached by Carmichael (1907). For accounts of the literature reference may be made to these papers.

Since, however, certain of the conclusions arrived at are still matters of controversy,* it has been thought worth while to place on record the results of further experiments upon rabbits, dealing, firstly, with the effects of ovariotomy in full-grown adults at different periods after the operation, and secondly, with the effects of the same operation in young animals.

It has been suggested that the changes occurring in the uterus may be due to an interference with the blood supply to that organ, in the removal of the ovary. The uterus in the rabbit, as in the human subject, has an independent vessel of supply derived from one of the pelvic vessels, and it is inconceivable that any operation undertaken with care for the removal of the ovaries, situated as they are high up in the abdominal cavity, could interfere with this vessel. Care was also taken in our experiments to avoid injuring the anastomotic branch of the ovarian artery, which passes along the Fallopian tube to join the uterine.

For this purpose in several of the experiments a lateral incision was made over the ovary on each side, so as to obtain better access and interfere with the mesovarium as little as possible by traction, a condition which is unavoidable in removal of the ovaries by a mesial incision. Thus all dangers of fallacy from vascular interference were avoided.

A mesial incision is often unsatisfactory for the removal of ovaries, in that the upper pole of the ovary may be left behind in dividing its superior attachment, considerable traction being required to expose the organs freely.

In the following experiments fully grown rabbits were used:—

Experiment 1.—The ovaries were removed by a mesial incision, and the animal killed after an interval of three and a-half months. Sections showed that the uterine stroma had undergone considerable fibrosis and was reduced in amount. The glands had almost entirely disappeared. The superficial epithelium was, however, fairly normal, but degenerated in places, some of the cells being vacuolated. The muscular wall did not show much degeneration, but was somewhat thinner than usual. Superficially the uterus, whose size was carefully noted at the time of the operation, was perceptibly smaller.

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* See the latter part of the present paper, where recent theories upon the nature and importance of the uterine influence are discussed.

Experiment 2.—The ovaries were removed by a mesial incision, and the animal killed three and a-half months subsequently, as in the preceding experiment. The glands and epithelium did not show the same degree of degeneration as in the previous case. Some fibrosis was, however, clearly discernable throughout the stroma. The muscles appeared to be not well developed, but otherwise were fairly normal.

Experiment 3.—The ovaries were removed from a pregnant rabbit by two lateral incisions. Two days afterwards the rabbit aborted. After five and a-half months the rabbit was killed. The uterus appeared very pale and atrophic looking. Mammary tissue was almost non-existent. Adipose tissue was super-abundant. Sections through the uterus showed that the mucosa was very thin and also fibrotic, the nuclei of the stroma being few and far between, excepting in the immediate neighbourhood of the epithelium, just below which they were much more numerous. The glands were represented by mere traces. The epithelial cells were somewhat degenerated, not taking on the stain so well as normally. There were very few blood-vessels.

Experiment 4.—The ovaries of a rabbit were extirpated by means of two lateral incisions a week after parturition. The animal was kept alive for five and a-half months, during which time it received 22 injections of commercial ovarian extract, made to determine whether this would have the effect of arresting the uterine degeneration. The extract was injected intra-peritoneally, one injection on an average being made every week. It was found, however, that whereas sometimes the injection appeared to result on the following day in a congestion of the mucous membrane of the external generative organs, the atrophy of the uterus was in no way prevented. Sections showed a very thin fibrotic mucosa, and a thin muscular coat. As in the preceding case, the glands had almost disappeared, and many of the epithelial cells were vacuolated and obviously degenerate. In the cervix the changes were not so pronounced, the epithelial cells being nearly normal in appearance.

Experiment 5.—The ovaries were removed by two lateral incisions. The rabbit was killed six and a-half months subsequently. Very pronounced changes had taken place. The uterine stroma was extremely fibrotic, and contained no glands. The epithelium was much attenuated, the nuclei having failed to take up the stain to the usual extent. A few very small capillaries, however, could be still seen in the stroma. The muscle fibres were much broken up. The cervix had not undergone the same amount of degeneration, but some of the epithelial cells appeared vacuolated.

In the following two experiments very young immature rabbits were used :—

Experiment 1.—The ovaries were removed on May 29. The rabbit weighed 660 grammes. On August 8 it weighed 1640 grammes. On March 20 the rabbit was killed after first being weighed. The weight was 3450 grammes, the animal being fully grown and in perfect health. The uterus was found to be absolutely infantile, being no bigger than a rat's. Sections showed that the muscular wall was almost totally undeveloped. The mucosa was somewhat thicker, but fibrous throughout. The lining epithelium was, however, present.

Experiment 2.—The ovaries were removed from a very small rabbit on June 5. The animal weighed 740 grammes. On August 8 it weighed 1450 grammes. On March 20 it was killed after being first weighed. The weight at that time was 2080 grammes. The rabbit was quite fully grown and very fat. The uterus, as in the preceding experiment, was very small, being not so large as an adult uterus after the removal of the ovaries some months previously, as shown by our first series of observations.

These experiments show that the removal of the ovaries at an early period of life, while arresting the development of the other generative organs, has no effect on the growth and general health. The weights, as given above, compare favourably with those of unoperated rabbits and with those of animals in which the uterus had been removed (see below).

Influence of the Uterus upon the Ovaries.

Although the bulk of evidence brought forward clinically seems to show that the uterine functions fall into abeyance after removal of the ovaries, a considerable number of cases have been reported in which menstruation has persisted after oöphorectomy. Pozzi (1905) has recently recorded several such cases, but the uterus in the majority contained a uterine fibroid or other pathological condition.

The explanation of such cases has always been a difficult one, but it has probably led to the ideas now held by some surgeons that the uterus is capable of performing its functions independently of the ovaries, and even that the functions of the ovaries themselves depend largely on the presence of the uterus and its secretions.

Zweifel and Abel, in a communication to the German Gynæcological Congress (1899) in tracing the after-histories of cases of hysterectomy, found that when the body of the uterus was removed, entire atrophy of the ovaries always followed, so that menopausal symptoms set in similar to those occurring after oöphorectomy. In three cases, however, in which a portion of the uterine mucous membrane was conserved, menstruation persisted and menopausal symptoms were absent.

Doran (1905), after following up the after-history of 60 cases of sub-total hysterectomy, is inclined to support Abel and Zweifel, in that the uterus should be removed above the cervix, in order to prevent sudden artificial menopause, while he also cites two cases where menstruation persisted after removal of the body of the uterus and both ovaries, the cervix being left behind.

Mandl and Bürger (1904) in an exhaustive monograph on the effects of hysterectomy with and without the ovaries hold that in those cases in which the ovaries are left behind there is a gradual cessation of ovarian function due to atrophy or degeneration of the ovaries.

Holzbach (1906), however, states that, as a rule, the ovaries do not degenerate after hysterectomy, and that where such degeneration does occur it is probably due to the interference with the nervous connections consequent upon the operation of removal.

Blair Bell (1906) holds that the theory of the internal secretion of the ovary is inconclusive, and that the clinical facts go to show that menstruation is due to a uterine secretion, and the menopause due to its absence, while he supposes ovulation itself to depend on the circulation of this secretion, which he calls "uterin." He mentions that there have been many cases in the Royal Infirmary at Liverpool in which ordinary menstruation continued after the removal of the ovaries, and makes further statements pointing to the conclusion that the ovaries invariably atrophy after the removal of the uterus.

Bond (1906), on the other hand, believes that the ovarian secretion is influenced by a saline secretion from the endometrium, the two, however, acting antagonistically to one another, so that prevention of the uterine secretion by hysterectomy favours hypertrophy of the ovary. Bond's view, therefore, is diametrically opposite to that of Blair Bell.

Bond records two experiments on the results of hysterectomy. In one of these the entire uterus of a rabbit was removed and the animal killed after five months. Both ovaries were found to be normal. In the other experiment the left uterine cornu only was extirpated and the rabbit killed after five months. The ovaries in this case also were in no way atrophied. Bond expresses the belief that the prevention by previous hysterectomy of the secretion of the saline fluid by the endometrium of the anœstrous uterus favours the overgrowth of luteal tissue in the ovary.

Stress has also been laid upon the well-known fact that whereas the corpora lutea of the ovary continue to undergo development for a considerable period of time if pregnancy supervene after ovulation, this hypertrophy soon ceases in the absence of pregnancy. Bond records an experiment on a rabbit in which the ovary after being grafted to an abnormal position contained a somewhat aberrant "corpus luteum of pregnancy" in association with a gravid uterus. Such observations are regarded by him as affording evidence of an internal uterine secretion (differing from that elaborated by the ancœstrous uterus), acting on the ovaries and so inducing a growth of luteal tissue. It must be remembered, however, that the occurrence of pregnancy exercises a profound effect over the whole maternal organism, and not merely upon the condition of the ovaries.

Certain other authors have adopted views which imply a dependence of the ovaries upon some function of the uterus. Thus, Loewenthal (1884) appears to have held that ovulation occurs as a result of a stimulus in some way set up by the uterine changes of the procœstrum. Moreover, Heape (1900) has expressed the belief that œstrus (which depends normally upon the presence of the ovaries) "is possible only after the active changes due to procœstrum have taken place in the uterus," but this statement clearly applies to normal animals.

Excepting for the two experiments of Bond, which have just been referred to, no experimental work appears to have been published upon the effects of hysterectomy on the ovaries. The following experiments, therefore, notwithstanding the fact that the results reached were negative, are not without importance.

Experiment 1.—On April 8 the uterus was removed entire from a very young rabbit which weighed 420 grammes. On August 8 the weight was 1640 grammes. The rabbit was killed on November 11 after being weighed. The weight was then 2060 grammes, the animal being apparently fully grown. The ovaries were well developed, having increased greatly in size since the operation. On histological examination they were found to be normal, containing numerous growing Graafian follicles, and showing no signs whatever of degeneration.

Experiment 2.—On April 8, the uterus of a very young rabbit was removed, excepting the cervix. Weight, 390 grammes. On August 8 the weight was 1640 grammes. On November 11 the rabbit was killed. Weight, 1940 grammes. The ovaries were normal in every way.

Experiment 3.—On May 2 the uterus was removed entire from a young rabbit. Weight, 650 grammes. On August 8 the weight was 1660 grammes. The rabbit was put with the buck at intervals and noticed to copulate. On

January 20 it was killed when quite full grown and in perfect condition. Weight, 3020 grammes. The ovaries were extremely well developed and contained numerous discharged follicles.

Experiments 4 *and* 5.—In each experiment the uterus was removed from a very young rabbit, excepting the cervix, and the rabbit killed after several months (in the one case after eight months and in the other after 10 months). Œstrus was noticed in both cases after the animals had reached maturity. The ovaries in both cases were normal, those of one animal containing large follicles, and those of the other (which had been with the buck) recently ruptured follicles.

Experiment 6.—The uterus was removed entire from a very young rabbit on May 17. Weight, 470 grammes. In August the animal was observed to be ill and on August 28 was killed. The ovaries had undergone development since the operation and were normal in histological appearance.

Experiment 7.—The uterus and Fallopian tubes were completely removed from a young rabbit on December 9. The rabbit was killed on April 3, when the ovaries were found to be normal and to contain many follicles.

In four experiments on rats the uterus was removed entire in the month of July. Two of the rats were killed in January, when the ovaries showed no indications of degeneration. The other two were killed in April, the ovaries being large in size and containing many protruding follicles.

On the other hand, marked uterine degeneration has been recorded in rats after the removal of the ovaries for shorter intervals of time (Marshall and Jolly, 1907).

Further, in the case of a ferret in which the uterus was removed, the histological appearance of the ovaries was normal after three months, when the animal unfortunately died. The interval which had elapsed, however, was probably too short to admit of the deduction of definite conclusions.

It may be concluded as a result of these experiments that the growth and development of the ovaries are in no way dependent upon the presence of the uterus. Such a conclusion is no doubt in opposition to some of the clinical evidence, but it is one which on phylogenetic grounds might be expected, since the uterus is an organ which came into existence comparatively recently in the course of vertebrate evolution, whereas the ovary is common to all Metazoa.

It has been shown, further, that œstrus and coitus may occur after the complete removal of the uterus. Whether under such a condition the other generative organs undergo any of the changes which normally characterise the proœstrum was not determined, since such changes are in any case difficult to detect in rabbits.

It was likewise impossible to determine definitely whether there was any increase of ovarian activity consequent upon hysterectomy (such as one would infer if Bond's theory of an antagonistic uterine secretion were correct), but we failed to discover any clear indication of its existence.

Summary of Conclusions.

The results obtained by these experiments lead us to summarise our conclusions as follows:—

1. The removal of the ovaries in young animals (rodents) prevents the development of the uterus and Fallopian tubes. These remain in an infantile condition. The subsequent growth and general nutrition of the animals seem to be unaffected.

2. The removal of the ovaries in adult animals (rodents) leads to fibrous degeneration of the uterus and Fallopian tubes (most marked in the mucous membrane). The animals' subsequent health and nutrition remain good.

These observations, for the most part, support the evidence obtained clinically in the human subject after surgical operation.

3. The removal of the uterus in a young animal has no influence in preventing the further development of the ovaries. These are capable of ovulating and forming corpora lutea after adult life has been reached.

4. The removal of the uterus in an adult animal does not give rise to any degenerative change in the ovaries, if the vascular connections of the latter remain intact.

These latter observations do not support the contentions of those surgeons who advocate sub-total hysterectomy, believing that the functional activity of the ovary is in some way dependent on the presence of the uterus.

The expenses of this investigation were defrayed by grants from the Carnegie Trust for the Universities of Scotland.

LITERATURE.

Blair Bell, 'Liverpool Medico-Chirurgical Journal,' July, 1906.
Bond, 'Brit. Med. Journ.,' July 21, 1906.
Carmichael, 'Journ. Obstet. and Gynæc.,' March, 1907.
Doran, 'Lancet,' November 4, 1905.
Heape, 'Quart. Journ. Micr. Sci.,' vol. 44, November, 1900.
Holzbach, 'Arch. f. Gynäk.,' vol. 80, 1906.
Loewenthal, 'Arch. f. Gynäk.,' vol. 24, 1884.
Mandl and Bürger, 'Die Biologische Bedeutung der Eierstöcke nach Entfernung der Gebärmutter,' Leipzig, 1904.
Marshall and Jolly, 'Roy. Soc. Edin. Trans.,' vol. 45, 1907, and 'Edinburgh Med. Journ.,' March, 1907.
Pozzi, 'Annales de Gynéc. et d'Obstet.,' December, 1905.
Zweifel, 'Zentralb. f. Gynäk.,' No. 21, 1899.

The Ascent of Water in Trees: (Second Paper).

By A. J. Ewart, D.Sc. Lond., Professor of Botany, University of Melbourne.

(Communicated by Francis Darwin, For. Sec. R.S. Received April 18,—Read May 9, 1907.)

(Abstract.)

The physical conditions in the vessels interpose no obstacle to the exertion of a pumping action on their contents controlled by the medullary ray cells. There are many theoretical ways in which surface tension action could be brought into play in this manner, but no practical proof of the existence of any such action in the wood vessels has as yet been obtained. Similarly, the bleeding of roots and stems affords no absolute proof of the existence of a vital pumping action in them, since all the observed cases can be explained as the result of osmotic action coupled with a reabsorption of the osmotic materials from the ascending stream, and their surface adsorption by the walls of the vessels. Instances are given in which an apparent pumping action proved to be of this character.

Nevertheless, the experiments detailed or quoted in the above paper all tend to show that the continuous ascent of water is only possible in living wood, and that the power of conduction is rapidly lost on death, without any mechanical blocking of the vessels being necessarily responsible for the change. Hence we are forced to conclude that the living cells in tall trees continually restore the conditions for the ascent of water wherever these are affected by the excessive emptying of the vessels, and decrease the resistance to flow, as far as possible, by maintaining continuous water columns in parts at least of the wood. So long as these are present *ab initio*, a pumping action only becomes necessary in trees over 20 to 50 metres in height, but suspended columns cannot be maintained for any length of time in the vessels of tall trees without the aid of the living cells of the wood.

The energy required to pump water upwards in the tallest trees represents only a small fraction of that produced by the daily photosynthetic assimilation, and it is the feeble character and diffuseness of the pumping action which renders it so difficult to demonstrate practically.

Contrary to Strasburger's statement, no vessels appear to run as open channels from end to end of any tree, the longest vessel observed being 564 cm. as measured by the length of stem in which it ran (*Wistaria*). In this plant, however, and others also, owing to the irregular course followed by

the vessels, these are always longer, and sometimes considerably longer, than the piece of stem in which they occur.

Experiments on the suction and exudation of trees at different levels, and upon the influence of the entry of air and water under pressure, showed that no continuous suspended water columns, or high internal tensions, existed in the conducting elements of the trees experimented on (Maple and Poplar) during active transpiration, or, indeed, at any period of the year.

The same was shown by direct measurements of the pressure in intact vessels of *Wistaria* during active transpiration. This fact, coupled with the high total resistance to flow, shows that this resistance is overcome locally from point to point, and not by any enormous tension from above or pressure from below, neither of which exists, nor could be maintained to a sufficient extent to carry on the elevation of water in a tall tree. A high tension from above leads to rapid blocking with air; a high pressure from below leads to great loss by lateral exudation from the vessels.

The apparent differences of osmotic pressure previously observed between leaves at the base and apex of a tall tree are not evidence of the existence of any greater osmotic suction at the higher level, but are the result of the different ages and sizes of the cells, and of the different conditions to which they have been exposed. Equally great differences may exist between leaf-cells at the same level.

The surface adsorption of dissolved solids in the vessels plays a very important part in their function as translocatory channels, causing a delay in the ascent of dissolved solids, such as sugar and salts, and an accumulation of them along the outer walls of the vessels. The latter facilitates their outward diffusion, but at the same time renders the transference of small quantities of material between widely removed organs difficult or impossible.

The tallest trees in Australia do not appreciably exceed 300 feet in height, so that the values previously given for the maximal total resistance to the upward flow of sap in actively transpiring trees must be reduced to between 30 and 50 atmospheres.

In a Maple tree whose younger wood had been killed by formalin the transpiration current turned to the older, partially blocked, but still living wood, and none of Strasburger's experiments show definitely that efficient conduction is possible in the dead wood of tall trees. The ascent of water is, therefore, a vital problem, in so far as it depends upon conditions which hitherto can only be maintained in living wood.

The Rôle *of the Various Elements in the Development and Regeneration of Bone.*

By Sir WILLIAM MACEWEN, F.R.S.

(Received and Read January 24, 1907.)

(Abstract.)

The present inquiry has been undertaken with the view of obtaining data, chiefly by direct experiment, as to the *rôle* which the various elements play in the development and reproduction of bone. This communication deals with a part of the subject, under two heads:—A, the potentiality of the periosteum as a factor in the production of bone, and B, the regeneration of bone from proliferation of osseous tissue. The following is a brief *résumé* of the paper:—

A. POTENTIALITY OF PERIOSTEUM AS A FACTOR IN REPRODUCTION OF BONE.

(1) To test this, a complete cylinder, constituting a portion of the shaft of a long bone, was removed while the periosteum was preserved intact. This showed, ten weeks afterwards, an osseous defect, constituting a gap in the continuity of the shaft.

(2) Periosteum free from osseous plaques was removed and transplanted. This was not followed by reproduction of bone, but by absorption of the periosteum.

(3) Duhamel's silver ring experiments are discussed and the correctness of the deductions drawn therefrom are questioned.

In order to test whether the bone cells or the periosteum produces the bone which covers the silver rings, three experiments, each differing from the other, were performed, in which silver rings were placed on bone deprived of its periosteum, with the result that in each case the rings became covered with bone.

B. THE REGENERATION OF BONE, FROM PROLIFERATION OF OSSEOUS TISSUE.

The periosteum is shown to be a limiting membrane controlling the osteoblasts, as illustrated in fractures, when the periosteum is intact and when it is torn. The production of callus is not inherently greater in the lower animals than in man—the amount of callus in both depends on the limitation of the periosteum and the amount of movement. (4) Direct experiment, showing that a long bone deprived of its periosteum continues to grow, (5) so also do the flat bones of the skull. (6) Bone may be made

to grow in the midst of lacerated muscles by the mechanical distribution of osteoblasts. Observation. Riders Bone. Suggestion as to the production of myositis ossificans.

(7) Can shavings of nude bone grow on being placed between muscles in a gap in the continuity of the shaft? Experiment and result seven weeks after show that not only do they grow, but that they also proliferate to a very marked extent.

Is there any direct evidence to show that transplanted living bone actually grows and proliferates instead of forming, like blood clot, a passive framework for the granulation tissue to penetrate, which framework will then become absorbed? There is, as may be illustrated (8) in an instance of bone grown in sponge filled with granulation tissue.

(9) To test the osteogenic power of bone cells constituting the shaft of a long bone, they were grown inside of a glass tube. Result.

(10) Intra-human transplantation of bone. Result 28 years after. Data are obtained from this experiment as to the growth of the humerus from the proximal and distal epiphyseal cartilages respectively. Also as to interstitial osseous increase—evidence of the increase in length of the diaphysis from the epiphyseal cartilage toward which the nutrient vessel runs.

An Experimental Enquiry into the Nature of the Substance in Serum which influences Phagocytosis.

(Second Communication.)

By George Dean, M.A., C.M., M.B., Chief Bacteriologist, Lister Institute.

(Communicated by Dr. C. J. Martin, F.R.S. Received April 23,—Read May 30, 1907.)

In a previous communication it was shown that in certain immune sera, *e.g.*, in anti-staphylococcus, in anti-dysentery, and in anti-typhoid serum, there are present thermostable substances which, by their action, prepare the homologous organisms for phagocytosis. The thermostability in the case of these immune sera could be demonstrated by the use of Wright and Douglas' method, whereas in the case of normal serum another method was, as a rule, necessary. In the case of the serum of the horse, however, it was pointed out that even by Wright and Douglas' method the increase of phagocytosis, observed by using normal heated serum, was, as compared with salt solution, generally considerable.

In view of certain experimental data and having regard to Ehrlich's theory, the conclusion was arrived at that the thermostable substances of normal and immune sera were probably identical.

It was further shown that, when submitted to a temperature of 60° C., both immune and normal serum suffered a rapid and sudden diminution in opsonic power. This change occurs in the first few minutes of heating, after which further loss appears to take place very slowly.

The results obtained suggested that the sensitising action of the serum might be due to the combined action of two substances, the one thermostable, the other thermolabile; the one of the nature of the "substance sensibilisatrice," the other of the nature of an alexin.

The present paper is an account of certain experiments carried out with the view of elucidating this point.*

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* In the course of writing this paper, a communication by Neufeld and Hüne has reached me, in which, in a criticism of my interpretation of these curves, they appear to have overlooked the fact that I had indicated the possibility that the sudden fall may be due to a destruction of complement. As at the time of the publication of my previous communication I had no experimental data to support that view, I preferred to leave the matter for further investigation. What I said was:

"Whether free complement may take part in the preparation of the microbe is difficult to determine. From the experiments detailed in this paper it is certain that it is not a necessary participant in this action. At the same time it is not improbable that the

THE INFLUENCE OF THE STRENGTH OF THE EMULSION UPON THE NUMBER OF ORGANISMS TAKEN UP BY THE LEUCOCYTES.

In Table I are shown the results obtained in two experiments carried out in the usual way by mixing one volume of serum, one volume of washed blood corpuscles and one volume of bacterial emulsion, putting the pipettes containing the mixtures in the incubator at 35°·5 C. for 15 minutes, after which the preparations for microscopical examination were made. The number of phagocytosed bacteria were subsequently counted, and the average number per leucocyte taken as the phagocytic index. As a rule, the number of organisms in 50 to 80 leucocytes were counted, and in many of the experiments the mean of two or three such counts by independent observers was taken. The emulsion of *Staphylococcus pyogenes aureus* was employed in different concentrations, full strength, half strength, quarter strength, one-eighth strength, and one-sixteenth strength.

Table I.

Emulsion of cocci concentration.	Average number of cocci phagocytosed in the case of—	
	Thick emulsion.	Thin emulsion.
1	86·0	16·5
$\frac{1}{2}$	52·5	9·6
$\frac{1}{4}$	17·4	6·4
$\frac{1}{8}$	7·9	2·9
$\frac{1}{16}$	3·0	1·3

The number of organisms taken up is, within the error of experiment, directly proportional to the concentration of the bacterial emulsion employed.

INFLUENCE OF DILUTION OF THE SERUM UPON THE NUMBER OF ORGANISMS TAKEN UP BY THE LEUCOCYTES.

When in similar experiments different concentrations of the same normal serum are employed, no direct proportionality can be demonstrated to exist between the serum concentration and the number of microbes phagocytosed. For example, the number of ingested organisms may be as great, or even greater, with the half, quarter, or even the one-eighth concentration, as with the full

immune body, when aided by complement, may act more powerfully, and that the sudden fall in the "opsonic" power of both normal and immune serum, on heating, is due to the destruction of the complement. I may revert to this subject on another occasion."

strength serum. This appears in a table in Wright and Douglas' (1903) paper, but no remark is made on the significance of the phenomenon.

The normal serum of several species of animal tested behaved in a similar manner in respect to dilution, as appears in Table II.

Table II.—Effect of Dilution of Normal Serum of Different Species. The Numbers indicate the Average Number of Micro-organisms taken up per Leucocyte.

Serum	Human serum and leucocytes.	Horse's serum and leucocytes.	Pig's serum and leucocytes.	Rabbit's serum and human leucocytes.	Human serum and leucocytes.
Micro-organism	Staphylococcus.	Staphylococcus.	Staphylococcus.	Staphylococcus.	Tubercle bacillus.
Concentration of serum—					
1	9·7	23·7	12·7	16·4	8·3
$\frac{1}{2}$	9·6	28·5	15·8	17·0	12·2
$\frac{1}{4}$	10·0	14·6	14·9	12·6	9·5
$\frac{1}{8}$	8·2	13·0	12·3	9·2	5·3
$\frac{1}{16}$	8·5	11·9	5·2	3·0	4·8
$\frac{1}{32}$	6·4	6·8	6·7	2·4	2·5
$\frac{1}{64}$	2·3	—	—	—	1·6

In addition to the experiments with staphylococcus, an experiment with the tubercle bacillus was also made, which shows that this relation is not limited to one micro-organism. Five dilution experiments on normal human serum, the diluent being normal salt solution, and the organism being the *Staphylococcus pyogenes aureus*, have been made.

The mean figures of these experiments for the pure serum, and the successive dilutions down to 1 in 32, if expressed in terms of unity are,

$$1,\quad 1{\cdot}16,\quad 1{\cdot}02,\quad 0{\cdot}71,\quad 0{\cdot}53,\quad 0{\cdot}35,$$

and the corresponding concentrations of serum,

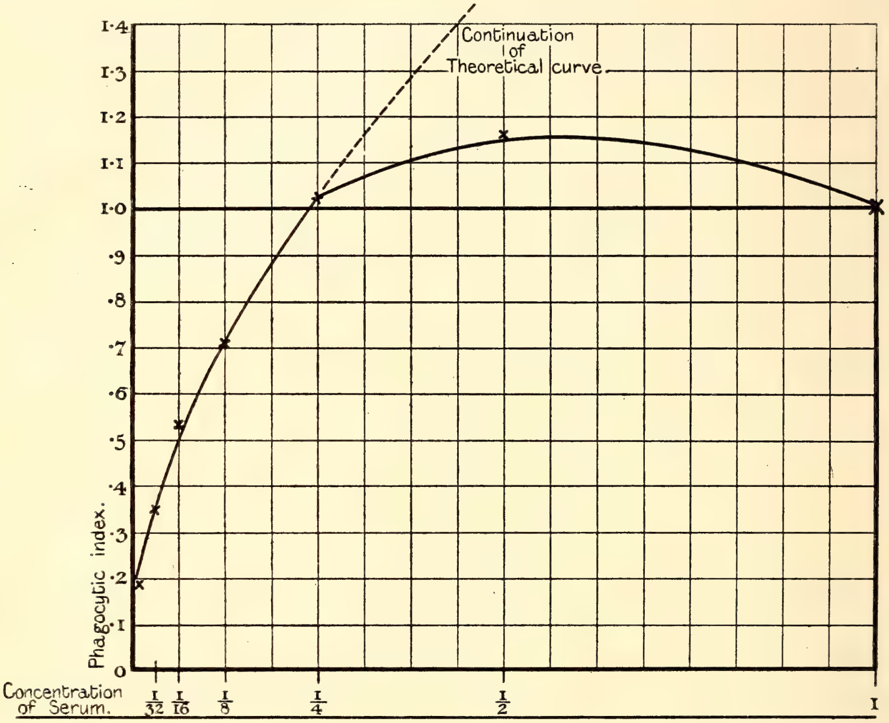
$$1,\quad 0{\cdot}5,\quad 0{\cdot}25,\quad 0{\cdot}125,\quad 0{\cdot}0625,\quad 0{\cdot}03125.$$

I am indebted to Dr. J. C. G. Ledingham for pointing out to me that after the second number there exists an approximate constant ratio between the second series and the squares of the first, as shown below:

$$\begin{aligned}(1{\cdot}02)^2 &= 4{\cdot}2\ \ (0{\cdot}25),\\ (0{\cdot}71)^2 &= 4{\cdot}03\ (0{\cdot}125),\\ (0{\cdot}53)^2 &= 4{\cdot}4\ \ (0{\cdot}0625),\\ (0{\cdot}35)^2 &= 3{\cdot}9\ \ (0{\cdot}03125),\end{aligned}$$

and that if the points (0·25, 1·02), (0·125, 0·71), (0·0625, 0·53), (0·03125, 0·35) are plotted, they are found to satisfy almost exactly the parabola whose equation is $y^2 = Kx$, where K = 4·1 (mean of 4·1, 4·03, 4·4, 3·9). The points (0·5, 1·16) and (1·1) obviously do not lie on the parabola.

The curve shown in the figure has been drawn from the mean of the figures for each dilution.



It will be seen that with the half concentration of serum there is a slight rise in the curve. This occurs so consistently that it can hardly be due to experimental error. From the quarter concentration onwards a fall is seen in the curve.

It is interesting to note that this square-root proportionality is analogous to what was found by Schutz (1885), Volhard (1901) and others working with albumin-pepsin and fat steapsine, etc. If these data are sufficiently accurate to be relied upon, it means that the phagocytosis is proportional to the square root of the dilution of serum for these concentrations, but that another factor is probably at work to account for the upper part of the curve.

The importance of this question of dilution in relation to the estimation of the amount of sensitising substances in the serum in normal, pathological, and immune conditions is obvious.

A striking instance may be given here. One specimen of an anti-staphylococcic horse serum, when diluted 32 times with saline, was found to have lost only 25 per cent. of its sensitising power, whereas the corresponding dilution of a normal serum of the same age had lost 90 per cent.

This subject in its various aspects will be dealt with more fully upon a future occasion.*†

THE INFLUENCE OF THE ADDITION OF FRESH SERUM UPON THE OPSONIC POWER OF HEATED IMMUNE SERUM.

As was pointed out in my previous communication, thermostable immune substance is capable of sensitising the microbes for phagocytosis in the absence of free complement. Metchnikoff's view, that in such cases the complement may be supplied by the leucocytes, is a possible interpretation, but will not be dealt with in the present paper. I propose to discuss here the question as to whether the heated immune substance in the presence of

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* Some misunderstanding (Wright and Reid, 'Roy. Soc. Proc.,' 1906) has arisen in regard to my remarks ('Roy. Soc. Proc.,' vol. 76, p. 506) on the limitations of Wright and Douglas' technique. I would again point out that their method may fail to demonstrate sensitising substances where these are present in considerable amount, and may show no difference between two fluids, notwithstanding that one contains a large amount of sensitising substance, the other only a small amount, or none.

To illustrate this, I may quote some experiments which were carried out with the view of determining the limits of sensitising action in an immune staphylococcic serum. Dilutions of 1 in 100 of this immune serum, and 1 in 100 normal serum, when tested by the ordinary technique of Wright and Douglas, showed no difference in their action, which was practically *nil*. On the other hand, if one mixed a volume of these dilutions with an equal volume of a coccal emulsion, so that the ultimate dilution was 1 in 200, and placed the mixtures at 35°·5 C. for one hour, and, after re-emulsifying both, carried out the ordinary technique, a very marked difference could be determined. The results obtained with the same sera, at an interval of some months, by three different observers, who endeavoured to adhere to standard conditions as to emulsions, etc., were as follows :—

	Phagocytic index of	
	Normal serum.	Immune staphylococcic serum.
(1)	2·4	21·0
(2)	3·3	18·7
(3)	1·9	23·7

† After I had made a communication on the effect of dilution at the Pathological Society of Great Britain and Ireland, a paper by Simon, Lamar, and Bispham, which deals with the importance of this subject of dilution, came into my hands.

fresh serum produces a greater effect than results from the two substances acting independently; in other words, is the action similar to, or identical with, the action of a bacteriolytic amboceptor and complement? At least two methods may be employed in the study of this problem: one, the direct method, of estimating the activity of the bodies when acting separately and in combination; the other, indirect, by employing something which will throw out of action one of the constituents of a combination, leaving the other unaffected.

THE DIRECT METHOD.

Experiments with Staphylococci.

The ordinary technique was employed. In this experiment 1 volume of leucocytes, 1 volume of coccal emulsion and 1 volume of the serum were employed. The result was as follows:—

	Average cocci per leucocyte.
Normal serum of rabbit, fresh, 1 volume	11·9
Immune serum of rabbit, heated to 60° C. for 20 mins., 1 volume	7·1
1 part immune heated and 4 parts fresh normal serum, 1 volume	33·0

The calculated value of the mixture at the highest estimation does not exceed 19, whereas the experimental value is 33. Some other factor must therefore be at work: in this experiment probably a complementing of the immune heated serum by the fresh serum.

Another experiment of the same type may be recorded in which a slightly different technique was employed.

	Average cocci per leucocyte.
Normal rabbit serum, heated, 1 volume, + Immune staphylococcus rabbit serum, heated, 1 volume, + Normal salt solution, 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	3·0
Normal rabbit serum, heated, 1 volume, + Fresh normal rabbit serum, 1 volume, + Normal salt solution, 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	6·9
Normal rabbit serum, heated, 1 volume,* + Immune staphylococcus rabbit serum, heated, 1 volume, + Normal rabbit serum, fresh, 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	15·1

* This was added to control a factor in another experiment.

Here the calculated value does not exceed 10, whereas the experimental value was 15.

Experiments with the Dysentery Bacillus of Shiga.

In the case of such organisms as the Dysentery bacillus, Typhoid bacillus, and Cholera vibrio, a difficulty presents itself owing to the occurrence of bacteriolysis. If the full strength of certain fresh normal sera is employed, an accurate estimation of the phagocytosis by this method of investigation may be impossible on account of the fact that many of the bacilli disappear altogether or are visible only as shadow forms both inside and outside the leucocytes. These phenomena are still more pronounced when one mixes with such a fresh normal serum a heated immune serum of any potency. The intracorpuscular and extracorpuscular bacteriolysis in such an experiment may be extreme. By a process of dilution of both the normal and the immune serum it was found possible to overcome this difficulty. For example, after a number of orientating experiments with dysentery, cholera, etc., it was found that if a dilution of 1/100 of a particular sample of heated anti-dysenteric serum was employed with a dilution of 1/20 or 1/30 of fresh serum, marked phagocytosis occurred with slight intracellular but no obvious extracellular bacteriolysis. When 1 volume of these dilutions is mixed with 1 volume of leucocytes and 1 volume of bacterial emulsion, the ultimate dilutions reached are 1/60 or 1/90 of the fresh normal serum and 1/300 of the heated immune serum.

The procedure carried out in such an experiment is as follows. Tubes and preparations are made in the usual manner from the following combinations:—

	Average number of bacilli per leucocyte.
A. Heated anti-dysentery serum (dilution 1 : 100), 1 volume, + Normal salt solution, 1 volume, + Leucocytes, 1 volume, + Dysentery bacillus (Shiga), 1 volume.	4·3
B. Fresh normal serum (dilution 1 : 30), 1 volume + Normal salt solution, 1 volume, + Leucocytes, 1 volume, + Dysentery bacilli (Shiga), 1 volume.	5·6
C. Heated anti-dysentery serum (dilution 1 : 100), 1 volume, + Fresh normal serum (dilution 1 : 30), 1 volume, + Leucocytes, 1 volume, + Dysentery bacilli, 1 volume.	16·1

In Table III are shown the results of four such experiments with anti-dysentery serum and fresh normal sera of different ages, with the mean values obtained.

Table III.—Average Number of Bacilli Ingested per Leucocyte.

Experiment number.	A. Normal serum fresh. Dil. 1 : 30, ultimate dil. 1 : 90.	B. Anti-dysentery serum heated to 60° C. for 20 minutes. Dil. 1 : 100, ultimate dil. 1 : 300.	C. A mixed with B.
(1)	1·7	1·0	7·5
(2)	0·5	1·2	5·9
(3)	5·6	4·3	16·1
(4)	2·5	1·3	9·0
Total	10·3	7·8	38·5
Mean of averages	2·5	1·9	9·6

If the effect produced by the combined action of the normal fresh serum and the immune heated serum were merely additive, the calculated value should be 4·2, whereas the experimental value is 9·6. Taking into consideration the results obtained in the experiments upon the influence of dilution, we must assume that the mixture containing the higher serum concentrations should have given a lower count even than 4·2, whereas, by experiment, a count of 9·6 is obtained. Another factor, therefore, must be at work in the production of this result, in all probability the complementary of the immune serum by the fresh normal serum.

Experiments with the Typhoid Bacillus and the normal Sera of the Guinea-pig and of the Rabbit.

Certain normal sera have been very thoroughly investigated for the presence of amboceptor and complement in connection with bacteriolysis. It seemed of interest to examine some of these for sensitising and complementing action from the standpoint of phagocytosis.

If a like relation is proved to exist, it will be at least an indication that the substances which prepare the microbes on the one hand for extracellular bacteriolysis, and on the other hand for phagocytosis, are identical, and that free complement, while an essential factor only in the former process, yet plays in the latter also an important, if secondary, *rôle*.

The sera of the rabbit and guinea-pig, which have been investigated by Wechsberg (1902) and others for amboceptor and complement action in relation to the bacteriolysis of the typhoid bacillus, offer excellent material for a comparative study of the two processes.

The following experiment, which is a repetition and confirmation of an experiment of Wechsberg, shows how the matter stands for bacteriolysis in the case of the guinea-pig and of the rabbit.

Experiment.—A 24-hours' agar culture of a typhoid bacillus, recently isolated from the blood of a patient, was emulsified in bouillon and diluted. Into each of a series of small test-tubes there was introduced a quantity corresponding to 1/1000 of the whole bacterial mass. Serum was added in varying amount, and the volume in each tube made the same by the addition of normal salt solution. A small volume of broth was also added to each tube.

These tubes were then placed in the incubator for three hours, after which a fixed volume was taken from each and plated.

At the same time the various serum constituents were tested for sterility.

Table IV

Culture.	Fresh unheated guinea-pig's serum.	Normal rabbit's serum heated to 60° for 20 minutes.	Number of colonies on agar plate.
	c.c.	c.c.	
$\frac{1}{10000}$ of an agar culture 1 day old of *B. typhosus*	0·5	0	109,998
	0·25	0	uncountable
	0·1	0	uncountable
	0·5	1·0	4,365
	0·25	1·0	21,604
	0·1	1·0	uncountable
	0·0	1·0	uncountable

This experiment shows that inactivated rabbit's serum is capable of being reactivated by normal guinea-pig's serum, so that the combination of the two is capable of producing a marked bacteriolytic effect in quantities which, acting apart, have little or no such action. Comparative tests with fresh rabbit's serum and fresh guinea-pig's serum showed that the latter had much less bacteriolytic action than the former, and the above experiment proves that this difference is due to a deficiency of amboceptor.

Employing the opsonin technique, a number of tests was performed with the object of ascertaining how these two sera influenced the bacilli in regard to phagocytosis.

Experiment.		Phagocytic index.
1	Guinea-pig's serum, heated to 60° for 20 minutes	0·2
2	Rabbit's serum, heated to 60° for 20 minutes	0·4
3	Guinea-pig's serum, fresh	5·7
4	Rabbit's serum, fresh	10·5
5	Rabbit's serum (60° for 20 minutes) + guinea-pig's serum, fresh	15·6

All were brought to the same volume.

A repetition of Experiments 3, 4, and 5, with a thinner emulsion of the typhoid bacillus, gave the relation 0·9, 2·0, and 3·6.

These experiments show, in the case of the sera of the rabbit and of the guinea-pig in relation to the typhoid bacillus, a marked parallelism between the extra-corpuscular bacteriolytic function and the sensitising or opsonising function by means of which the organisms are prepared for phagocytosis, and that a complementary action takes place in both cases.

The Indirect Method.

These experiments were based on the observations made by Ehrlich, Morgenroth, Bordet, Pfeiffer, and others that the injection of the serum of one species into another gives rise to the elaboration in the blood of the latter of anti-bodies, which may act either on the amboceptor or on the complement. Anti-complement formation is a common phenomenon, whereas anti-amboceptor is of rare occurrence. The mechanism of the action of such sera has been the subject of much discussion which cannot be touched on here. In the present paper the term "anti-complement serum" will be used without prejudice, the possibility that such a serum may act on other bodies besides complement not being overlooked. In relation to the present subject, it seemed of interest and importance to ascertain the effect produced by an anti-complement serum in experiments of the type of these with which we are dealing. Whilst these experiments were in progress, Muir (1906) published results obtained by a similar method with which, in the main, the present series are in agreement.

Technique Employed.

The anti-complement serum was prepared by injecting a rabbit on several occasions with human or horse serum which was previously heated to 58° to 60° C. for half an hour. The anti-complement serum, after separation, was itself heated to 60° C. for 20 minutes. Varying proportions of this serum were then added to the homologous fresh serum, or to the mixture of which it was a constituent part. The tubes containing these were incubated for two hours at 35°·5 C. In this way a precipitin reaction was produced. The supernatant fluid removed from the precipitum was employed in the experiment. By doing a series of eight or 12 such tests, the most effective combinations of the serum were ascertained. In some of the experiments there was no such preliminary treatment of the fresh serum by the anti-serum, this being simply made one of the constituent parts of the mixture of leucocytes, bacilli, etc., and allowed to act during 15 or 20 minutes. The further carrying out of the experiments was as follows:—

(A) Normal rabbit serum, heated to 60° C. for 20 minutes, 10 volumes, +
Fresh normal horse serum, 5 volumes.
(At 35°·5 C. for two hours.)

(B) Anti-complement (horse) serum of rabbit, heated to 60° C. for 20 minutes, 10 volumes, +
Fresh normal horse serum, 5 volumes.
(At 35°·5 C. for two hours.)

(C) Normal rabbit serum, heated to 60° C. for 20 minutes, 10 volumes, +
Normal horse serum, heated to 60° C. for 20 minutes, 5 volumes.
(At 35°·5 C. for two hours.)

(D) Anti-complement (horse) serum of rabbit, heated to 60° C. for 20 minutes, 10 volumes, +
Normal horse serum, heated to 60° C. for 20 minutes, 5 volumes.
(At 35°·5 C. for two hours.)

The supernatant fluid from each of the above mixtures A, B, C, and D, was subsequently mixed with an equal volume of human leucocytes and staphylococcic emulsion and kept at 37° C. for 15 minutes.

When horse serum had been injected into the rabbit to obtain the anti-complement serum, it was found impossible to use horse leucocytes in the experiment, on account of the rapid agglutination of the red cells and entanglement of the leucocytes. In this case, therefore, human leucocytes were used, and by so doing the possible danger of a certain amount of specific leuco-toxic action was avoided.

The results of seven experiments are detailed in Table V (p. 410).

It is seen that a great loss in the sensitising power has resulted in Experiment 2, where the anti-complement serum was used. The higher count of Experiment 4, as compared with Experiment 3, is probably largely due to the fact that the heated anti-complement serum had a higher sensitising power than the normal rabbit serum employed, the ratio being about 2 : 1. This last observation applies to all the corresponding figures in the table, which summarises the results of seven such experiments, with a varying proportion of anti-complement and normal sera *versus* fresh horse serum. On comparing the second and fourth columns of that table, it will be seen that fresh normal serum, after having been acted on by the anti-complement serum, gives almost the same phagocytic average as is obtained by heated horse serum under the same conditions, *i.e.*, a loss has occurred comparable to that produced by heating to 60° C. for 20 minutes.

Table V.—Results of Experiments with Anti-complement (Horse) Serum obtained from Rabbit, together with Control Results.

Number of experiment.	Fresh normal serum of horse, +		Serum of horse heated (60° for 20 mins.), +	
	A. Normal rabbit's serum. Heated to 60° C. for 20 mins.	B. Anti-complement serum of rabbit. Heated to 60° C. for 20 mins.	C. Normal serum of rabbit. Heated to 60° C. for 20 mins.	D. Anti-complement serum of rabbit. Heated to 60° C. for 20 mins.
1	17·5	7·5	4·1	5·5
2	10·7	3·5	1·2	3·4
3	8·3	2·1	2·6	0·8
4	8·4	1·1	1·1	3·2
5	6·3	0·6	1·3	0·2
6	7·1	4·4	1·4	3·0
7	6·0	2·5	1·0	1·1
Total	64·3	21·7	12·7	17·2
Mean of experiments	9·1	3·1	1·8	2·4

The serum of the horse, after having been heated for 20 minutes to 60° C., still shows some sensitising power, even when tested by Wright and Douglas' method. Several comparative tests by my assistants and myself, where a large number of leucocytes had been counted, show that heated normal horse serum, compared with normal salt solution, gave average counts in the ratio of about 4 : 1.

Action of Anti-Complement Serum in Complemented Mixtures.

The following experiments with staphylococci may be reported in full as the type of such experiments. The usual opsonic technique was employed, the constituent parts of the mixture introduced into the tubes being as follows (see table, p. 411).

A comparison of 1 and 4 shows that, by the action of anti-complement serum, the phagocytic average has been reduced from 15·1 to 2·4.

Similar experiments with the dysentery immune serum of the horse complemented with normal human serum showed, as the result of the action of the anti-complement serum, a reduction in the phagocytic index in one experiment from 7·5 to 1·3, in another from 5·9 to 1·7. In this case human anti-complement serum and horse leucocytes were employed.

	Phagocytic index.
1. Normal rabbit's serum, heated to 60° C. for 20 minutes, 1 volume, + Immune rabbit's staphylococcus serum, heated to 60° C. for 20 minutes, 1 volume, + Normal human serum, fresh (dilution 1 : 10), 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume	15·1
2. Normal rabbit's serum, heated to 60° C. for 20 minutes, 1 volume, + Normal salt solution, 1 volume, + Normal human serum, fresh (dilution 1 : 10), 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	6·9
3. Normal rabbit's serum, heated to 60° C. for 20 minutes, 1 volume, + Immune rabbit's staphylococcus serum, 1 volume, + Normal salt solution, 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	3·0
4. Anti-complement rabbit's serum, heated to 60° C. for 20 minutes, 1 volume, + Immune rabbit's staphylococcus serum, heated to 60° C. for 20 minutes, 1 volume, + Normal human serum, fresh (dilution 1 : 10), 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	2·4
5. Anti-complement rabbit's serum, heated to 60° C. for 20 minutes, 1 volume, + Normal salt solution, 1 volume, + Normal human serum, fresh (dilution 1 : 10), 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	1·7
6. Anti-complement rabbit's serum, heated to 60° C. for 20 minutes, 1 volume, + Immune rabbit's staphylococcus serum, heated to 60° C. for 20 minutes, 1 volume, + Normal salt solution, 1 volume, + Leucocytes, 1 volume, + Cocci, 1 volume.	3·7

Summary.

1. Dilution of fresh unheated serum is not accompanied, so far as the higher concentrations are concerned, by a fall in the sensitising power for certain organisms (staphylococcus, tubercle bacillus). The diminution in this was found, as a rule, to begin at the quarter concentration.

2. So far as the present experiments go, the points corresponding to the 1/4th, 1/8th, 1/16th, and 1/32nd dilutions of normal human serum lie on a parabola whose equation is $y^2 = 4X$, *i.e.*, for these dilutions the phagocytosis is proportional to the square root of the serum concentration.

3. The phagocytic index obtained by mixing appropriate dilutions of a heated immune serum with a normal fresh serum is greater than results from the two substances acting separately.

4. In the case of certain normal sera (guinea-pig and rabbit) previously investigated for amboceptor and complement in relation to the extra-corpuscular bacteriolysis of the typhoid bacillus (Wechsberg), an exact parallelism can be demonstrated to exist between that function and opsonisation. The normal amboceptor can be complemented by fresh serum in regard to both functions.

5. An "anti-complement" serum, when mixed with a fresh normal serum alone or in a mixture containing heated immune serum, throws out of action the thermolabile substance, whereas it does not appear to influence the thermostable substance.

REFERENCES.

Muir, R., and Martin, W. B. M. (1906), "On the Combining Properties of Opsonins of Normal Serum," 'Brit. Med. Journ.,' December 2, 1906.

Neufeld, F., and Hüne (1907), "Untersuchungen über baktericide Immunität und Phagocytoses," 'Arbeiten a. d. Kaiserlichen Gesund.,' vol. 25, Part 1.

Schütz (1885), 'Zeitschr. f. Physiol. Chemie,' von Hoppe-Seyler, "Eine Methode zur Bestimmung der relativen Pepsinmenge," vol. 9, p. 577.

Simon, C. E., Lamar, R. V., and Bispham, W. N. (1906), "A Contribution to the Study of the Opsonins," 'Journal of Experimental Medicine,' vol. 8, p. 651.

Volhard (1901), 'Zeitschr. f. Klin. Medizin,' "Ueber das fettspaltende Ferment des Magens," vol. 42, p. 414, vol. 43, p. 397.

Wechsberg, F. (1902), "Zur Lehre der natürlichen Immunität und über baktericide Heilsera," 'Zeitschr. f. Hygiene und Infections-Krankheiten,' vol. 39, p. 171.

Wright, A. E., and Douglas, S. R. (1903), "An Experimental Investigation of the *rôle* of Blood Fluids in connection with Phagocytosis," 'Roy. Soc. Proc.,' vol. 73, p. 357.

Wright, A. E., and Reid, T. (1906), "On Spontaneous Phagocytosis and on the Phagocytosis which is obtained with the Heated Serum of Patients who have responded to Tubercular Infection, or, as the case may be, to the Inoculation of a Tubercle Vaccine," 'Roy. Soc. Proc.,' B, vol. 77, p. 211.

CROONIAN LECTURE :—*On Globulins.*

By W. B. HARDY, M.A., F.R.S.

(Being the Croonian Lecture delivered May 25, 1905.—MS. Received April 9, 1907.)

Globulins are a class of proteids which occur in both animal and vegetable tissues. They are peculiar in the complexity of their relations to electrolytes. Insoluble in water, they are soluble in low concentrations of acids, alkalies, or neutral salts. In presence of acids the globulin is electro-positive, in presence of alkalies it is electro-negative, in presence of neutral salts it is electrically neutral. Electrically active globulin (*i.e.*, dissolved by acids or alkalies) is precipitated by minute amounts of neutral salts; also, no matter what its electrical state may be, or how dissolved, globulins are precipitated by neutral salts near the saturation point of the latter. The problem I propose to consider is their diversified relation to electrolytes.

Connected with this problem is another, namely, the relation of solutions of globulins to colloidal solution. Do they form hydrosols at all, and, if so, to what extent? Krafft urged the colloidal nature of soap solutions, because, within certain limits of temperature and concentration, they gelatinise, and have the vapour pressure of pure water. Kahlenberg and Schreiner, however, regard soap solutions as being crystalloid in character, because over the whole range of concentration the soap is a good electrolyte—it ionises and undergoes hydrolytic splitting, like other salts of a weak acid and strong base. Smits, again, by measurement of the vapour pressure over a wide range of concentration, is convinced that above a critical concentration the soap passes wholly into the colloidal state.

Exactly the same points arise in connection with proteid. Waymouth Reid has shown that the proteids in a solution exert no measurable influence upon the vapour pressure. On the other hand, solutions of globulins are relatively good conductors over the whole range of concentration through which it is possible to follow them. The dilution curve shows no break indicating a general change of state, even when concentration is pushed to the point where fluidity almost vanishes.

Again, colloids, as a class, are chemically inert. But globulins react actively with acids or alkalies to neutralise them. Globulin solutions, even at extreme concentration, form syrups and not true gels, except, perhaps, under special circumstances. Clearly, therefore, it is pertinent to ask whether globulins form colloidal solutions.

The globulin used throughout this research is that which is precipitated from ox serum by dilution and slight acidification.

Solution by Acids or Alkalies. Electro-positive or electro-negative globulin. When acid or alkali is gradually added to a suspension of globulin in water, the opaque suspension gradually changes to a clear transparent fluid. Between the opaque suspension, from which the globulin settles on standing, to the stage of limpid transparency there is no break. The most minute addition of acid or alkali (in the absence of salts) converts the suspension into a non-settling " solution " of low grade, which is opaque white from the large size of the particles of globulin dispersed throughout it. Further addition of acid or alkali raises the grade of solution. The globulin particles become smaller and smaller until complete transparency is reached.

The process can be reversed by dialysis, save that, at the lowest grade attainable, no precipitate settles in the absence of disturbing factors.

With the first addition of acid or alkali, and at the last stage of dialysis, the globulin is electrically active, so that it moves in an electric field, the direction of movement being that which one would expect if it combined with the acid or the alkali to form a salt, and the specific velocity is quite, or nearly, at its maximum value. A rise in the grade of solution is, on the whole, associated with a fall in the specific velocity, never in my experience with a rise in this value. The electric charge on the surface of the globulin particles therefore appears to reach maximal density when the solutions are still of exceedingly low grade.

These facts can best be explained by assuming that true salt formation occurs. This agrees with the view of all previous workers on the interaction of proteids with acids or alkalies (Sjöqvist, Bugarsky and Liebermann, and Cohnheim). Since globulins combine either with acids or bases, they have both an acid and a basic function—they are amphoteric electrolytes.

The globulin salts ionise in solution; therefore, in an electric field the entire mass of proteid moves. They also hydrolyse, but the hydrolysis offers special features resembling those which Jordis has pointed out in the case of sodium silicate, and Chevreul in the case of soaps. In both of these cases hyper-acid salts are formed, while hyper-acid salts are formed on dialysis of a solution of globulin by alkali, and hyper-basic salts on dialysis of a solution of globulin by acid.

By dialysis the degree of hyper-acidity or hyper-basicity can be raised, but, as Jordis finds in the case of silica, with continuously increasing difficulty. With the rise in the degree of hyper-acidity or hyper-basicity, the "grade" of solution diminishes, but, in the case of globulins, precipitation does not occur. Electrically active solutions of globulin cannot be precipitated by dialysis, nor at any stage do they cease to be electrically active.

If G be used to denote globulin, the equation of hydrolysis or dialysis would be:

$$x\,\mathrm{GHS} + y\,\mathrm{HOH} = (\mathrm{GHOH})_y\,(\mathrm{GHS})_{x-y} + y\,\mathrm{HS},$$

$$\text{or}\quad x\,\mathrm{GB} + y\,\mathrm{HOH} = (\mathrm{GH})_y\,(\mathrm{GB})_{x-y} + y\mathrm{BOH}.$$

In dialysis, the ratio x/y varies continuously. It is as indeterminate as the ratio between the combining salts has been found to be by Bödlander, Abegg, Sherrill, and others in the double salts of mercury and silver.

Clearly a relation of this kind agrees with van Bemmelen's definition of absorption compounds as chemical combination with variable composition.

Removal of water from a solution of acid or alkali globulin does not produce precipitation, and the dried gummy residue reabsorbs water and passes slowly again into a state of solution. Acid and alkali globulin, therefore, form, with water, solutions which have the feature characteristic of colloidal solutions, in that there are no saturation points.

In order to compare the solvent power of different acids or alkalies, it is necessary to fix upon some arbitrary point, such as the point of minimal opalescence. By the use of a system of controls and proper illumination this point furnishes very concordant values.

Measured in this way, it appears that for strong and medium acids solvent power is measured by the number of gramme molecules present, not by the number of gramme equivalents:

$$\mathrm{HCl} = \mathrm{H_2SO_4} = \mathrm{H_3PO_4}.$$

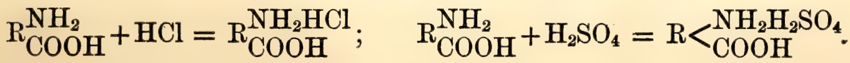
Very weak acids have a lower solvent power: $\mathrm{HCl} = 5\mathrm{H\bar{A}} = \pm 30{,}000\mathrm{H_3BoO_3}$.

These relations are explained by the very weak basic function of globulin. Salts with weak acids are much hydrolysed, and to reach the same grade of solution an excess of acid is needed in order to lower the degree of hydrolysis.

With alkalies, the weak alkali $\mathrm{NH_4OH}$ dissolves as well as the strong alkalies, owing to the fact that globulin acts as an acid of considerable strength.

The acid and basic functions were measured by the well-known methods—the catalysis of cane sugar and of methyl acetate—and the acid function found to be much the greater.

The molecular relation noticed above recalls the salts of amido acids:



$$\mathrm{R}\genfrac{}{}{0pt}{}{\mathrm{NH_2}}{\mathrm{COOH}} + \mathrm{HCl} = \mathrm{R}\genfrac{}{}{0pt}{}{\mathrm{NH_2HCl}}{\mathrm{COOH}};\qquad \mathrm{R}\genfrac{}{}{0pt}{}{\mathrm{NH_2}}{\mathrm{COOH}} + \mathrm{H_2SO_4} = \mathrm{R}{<}\genfrac{}{}{0pt}{}{\mathrm{NH_2H_2SO_4}}{\mathrm{COOH}}.$$

Data derived from measurements of electric conductivity, and from the behaviour with indicators, support the view that true salts are formed, and that the globulin acts much more strongly as an acid than as a base.

Direct Measurement of the Specific Velocity of Globulin "Ions."—This was carried out by the boundary method.

As the basic function of a globulin is weaker than its acid function, the salts GHS (globulin + acid) will be hydrolysed more than the salts GB (globulin + base). Therefore, in the equation of hydrolysis given above, y/x will be larger for the former than the latter. Similarly, comparing salts with $H\bar{A}$ and with HCl, y/x will be greater for the former.

Now with increase in the value of y/x the size of the proteid particles increases also. By dialysis, the size can be increased until the particles diffract white light. The growth in the case of solutions of sodium silicate can be traced by the appearance of molecular states incapable of passing through a parchment membrane. In a similar way, by continuous addition of AgCl to a solution of NaCl, molecular states are produced large enough to diffract white light.

In each of these cases these large molecules are ionic, that is to say, they take part in the electric transport. And their specific velocity is exceptionally high; that of $(AgCl)_x\ Cl_y$ being 57×10^{-5} at 18°, whereas Ag at infinite dilution is only 58.

"Ions" of this order of magnitude have the properties of matter in mass. Each is defined by a surface and moves under the influence of a surface contact difference of potential. With their appearance the fluid ceases to be homogeneous. It has internal surfaces. I propose to call such ions colloidal ions, or pseudoions. Their specific velocity is high, and, within wide limits, is independent of their size, and is controlled by the laws of electrical endosmose.

These conclusions are borne out by the boundary measurements and by the electrical conductivity of colloidal solutions.

For instance: theoretically the proportion of pseudoions in the following solutions of globulin should be:—

$$\text{In } H\bar{A} > \text{in HCl}, > \text{in NaOH},$$

and the specific velocities at 18° were found to be 23×10^{-5}, $11{\cdot}5 \times 10^{-5}$, $7{\cdot}6 \times 10^{-5}$. By Ostwald's law of the relation of specific ionic velocity to the number of atoms in an ion, the value 23×10^{-5} is an utterly impossible one for an ordinary ion of the magnitude of the proteid molecule.

Solution by Neutral Salts.—Globulins are dissolved by neutral salts owing to the formation of molecular compounds (G.BS). These compounds are readily decomposed by water with liberation of insoluble globulin

($G.BS + HOH = G.HOH + BS$). Therefore they are stable only in presence of a large excess of salt. Hence the solvent power of salts is from 200 to 500 times less than that of acids or alkalies. Hence, also, the presence of the globulin lowers the electric conductivity of the salt to only a small extent (2 to 6 per cent.).

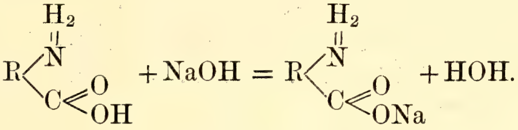
A double salt of the form AB.CD. ionises according to $AB.CD \rightleftarrows AB.C + D$. I have never succeeded in detecting any trace of such ionisation in the case of globulin and neutral salts; possibly owing to the extreme instability of the ions. The proteid does not move in an electric field, and it seems to take no part in the electric transport.

Owing to the insolubility of the dissociation products, the globulin can be precipitated from its solution with neutral salts by simple dilution. No degree of dilution will precipitate globulin from solution in dilute acid or alkali.

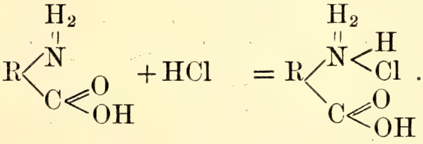
The compounds of globulin and alkalies (GB) are more readily dissolved by neutral salts than is simple globulin. Compounds of globulins and acids are insoluble by neutral salts, being decomposed with liberation of the acid.

If on the analogy of an amido acid a globulin combines owing to the presence of the NH_2 and the COOH group, then it forms salts with alkalies by replacement of hydrogen, and with acids by the change of the trivalent nitrogen of the amido group to the pentavalent form.

i.



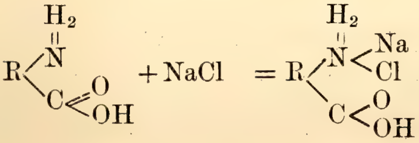
ii.



Where, then, does the neutral salt link on?

There are two possible places—the unsatisfied valencies of the nitrogen, or the unsatisfied valencies of the upper O of the COOH group. According to the oxonium theory, O has a maximum valency $O\equiv$, but $O\equiv$ is much less stable than $N\equiv$; therefore, one would expect the linkage to be by the amide group—

iii.



And this would account for the fact that the acid can be turned out of the combination with globulin by an excess of neutral salt, but the alkali cannot be.

It is easy to prove by the boundary method that the globulin, when dissolved by salts, takes no part in the electric transport. Dissociation of the salt-globulin compound by the water may be regarded as being practically completely suppressed by the excess salt present. This being the case, the diminution of the electric conductivity of the salt by the globulin may be used as a measure of the fraction of the salt actually combined with it.

In the following table a few values are given. Comparison is made between solutions containing the same amount of salt per litre; but in the one case the solution is saturated with globulin, in the other case it is a simple solution of the salt in water. No correction is made for the diminution of the molecular conductivity of the salt owing to replacement of a portion of the water by globulin, since it would amount to less than 0·01 per cent. No. III is of the nature of a control, to prove that electrolytic impurities adherent to the purified globulin may be neglected. The salt solution was made up with a dialysate in equilibrium with the suspension of globulin.

Serum centrifuged, diluted to 10 vols., globulin precipitated by $\overline{\text{HA}}$. The precipitate suspended in a large volume of distilled water and collected by the centrifuge, and re-suspended in water freed from gases by boiling.

Specific conductivity of the water employed, 6×10^{-6} rec. ohm, 18°.

I. Ox globulin suspended in water. 100 c.c. = 4·08 grammes dry globulin. Temperature, 18° :—

Salt.	Normality.	Specific conductivity, salt globulin, $\times 10^5$.	Specific conductivity, salt water, $\times 10^5$.	$\frac{\text{K salt globulin}}{\text{K salt water}}$.
K_2SO_4	0·1716	1445	1539	0·938
„	0·1679	1411	1506	0·937
NaCl	0·27	1850	1950	0·949

Specific resistance of the globulin suspension $3·1 \times 10^{-5}$.

II. Sheep globulin. 100 c.c. = 4·4 grammes :—

NaCl	0·2659	2145	2280	0·938
„		2150	2278	0·943

The measurements in II were duplicated in order to test the degree of accuracy of the measurements of volume.

III. Ox globulin dialysed until in equilibrium with water saturated with toluol :—

$MgSO_4$	0·11	517	543*	0·95

In this case the salt solution (*) was made up with the dialysate, not with water.

[*Note.*—In a recent paper* it is stated that globulin in solution does not alter the conductivity of the salt used to dissolve it. The conductivity of the salt "is the same as that of a similar solution in pure water." This statement is directly contrary to my own observations. It leads me to add a few words as to the precautions which are necessary in dealing with proteid solutions.

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* Mellanby, 'Journ. of Physiology,' vol. 33, p. 354, 1905.

Electrodes coated in the ordinary way with platinum black are quite useless. The proteid in their immediate neighbourhood is changed very rapidly and very drastically. An instance selected at random will show the magnitude of the effect.

18°. Globulin dissolved by dilute NaOH. 3·3 per cent. globulin. Readings at intervals of two minutes approximately :—

2560 ohms.
2542 „
2537 „
2530 „
2522 „
2538 „

The rise in the last observation followed upon slight shaking of the cell to wash the changed portion of the solution away from the electrodes. Platinum grey electrodes, prepared as Whetham directs, give no trouble. Readings with globulin solutions of all kinds remain quite constant after being for 48 hours and longer in contact with them.

Even with platinum grey electrodes, however, it is necessary so to adjust the cell that the observed resistance does not fall under about 500 ohms. With a lower resistance, and therefore a larger current, the readings become unsteady and too low.]

On the assumption that the whole of the drop in electric conductivity is due to association of the salt with the globulin, the above figures show that, at the particular concentration and temperature, 1 gramme of dry globulin combines with 33×10^{-5} gramme equivalent of NaCl and 26×10^{-5} gramme equivalent of K_2SO_4, while to dissolve 1 gramme of dry globulin 10×10^{-5} equivalent of alkali, or 18 to 36 equivalents of acid are needed. The figures for salt and acid are of the same order of magnitude, and this is what might be expected if salt and acid combine in the same way with the proteid.

APPENDIX.

(*Added December,* 1906.)

Viscosity of Solutions of Globulins.—The measurements of this value, very briefly touched upon in the lecture, have been amplified. They show the following interesting features :—

In dilute solutions of globulins the viscosity, as measured by the rate of flow through a capillary tube, is of the same order of magnitude for each of the three types—acid, alkali, and salt globulin.

With increasing concentration the viscosity increases, but the increase is much greater for alkaline globulin than for acid globulin, and for acid globulin than for salt globulin. The difference is very striking; thus, at a concentration of 7·59 grammes globulin per litre, the transpiration times are in the ratio—

Water	1
$MgSO_4$ globulin	4·66
HCl "	15·5
NaOH "	67·9

In solutions of salt globulin, the globulin particles do not carry electricity; "ionic" proteid is completely absent, therefore the higher viscosity of acid and alkaline globulin solutions is connected with the presence of "ionic" proteid, that is, of very large molecules carrying a charge.

Since globulin acts much more strongly as an acid than as a base, there will be in similar solutions of HCl globulin and NaOH globulin a greater concentration of "ionic" proteid in the latter. Here, again, increased concentration of "ionic" proteid goes with increased viscosity.

These conclusions agree with the observations of Reyher* on the viscosity of solutions of soap, and of Sackür† on solutions of the proteid caseinogen. It would, however, be rash to fix upon the simple proteid or soap ion as the agent. The effect of dilution, and of varying proportions of acid or alkali, make it possible that the high viscosity is due to the presence of the molecular complexes, which carry a surface charge, and which I have called "colloidal" ions, or pseudoions. Thus, further addition of small quantities of ammonia, beyond the amount necessary to dissolve the globulin to a transparent solution, *decreases* the viscosity. Ammonia is a very weak base, which, in solution, has a specific molecular conductivity much less than that of its compound with globulin. One cannot, therefore, refer the diminished viscosity to diminished ionisation of the ammonia-globulin compound, but the optical properties of the solution show that slight excess of ammonia does diminish the size of the particles of the globulin. It raises the "grade" of the solution.

A comparison of the different types of solution of globulin raises, in a special way, the question of the meaning to be attached to the phrase "colloidal solution." Mixtures of very diverse character have been classed as colloidal, some because they form jellies, others because they are physically heterogeneous, others because the osmotic pressure is exceedingly low, others, again, because the electric conductivity is abnormally low. Of these, perhaps, the only constant attribute is physical heterogeneity. Solutions which present any of the other distinctive features of the colloidal state always contain particles which are large enough to scatter light.

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* 'Zeits. f. Physik. Chem.,' vol. 2, p. 743, 1888.

† *Ibid.*, vol. 41, p. 672, 1903.

Amongst the attributes of colloidal solutions is the possession of an abnormally high viscosity. Judged in this respect, the salt globulin solutions are only slightly colloidal as compared with the solutions of alkali or acid globulin. They differ also from acid and alkali globulin in the relatively greater definiteness of the relations between the components. Dilution of a solution of salt globulin, for instance, brings about a sharp separation of solid and liquid. No such clean saturation points are found with the other two systems. The definiteness of the relations makes it possible to compare the system—neutral salt, water, globulin with other similar three-component systems.

Data sufficient for the purpose can be obtained from the measurement of the solubility of globulins in aqueous salt solutions made by Osborne and Harris,* and by Mellanby.† The curves which these authors give fail to show the true relations, since only two co-ordinates are used, namely for the salt and the globulin; the third component, water, is neglected. For the following curves, trilinear co-ordinates are used in the manner recommended by Stokes.

Fig. 1 shows curves plotted to scale from values derived from Osborne and

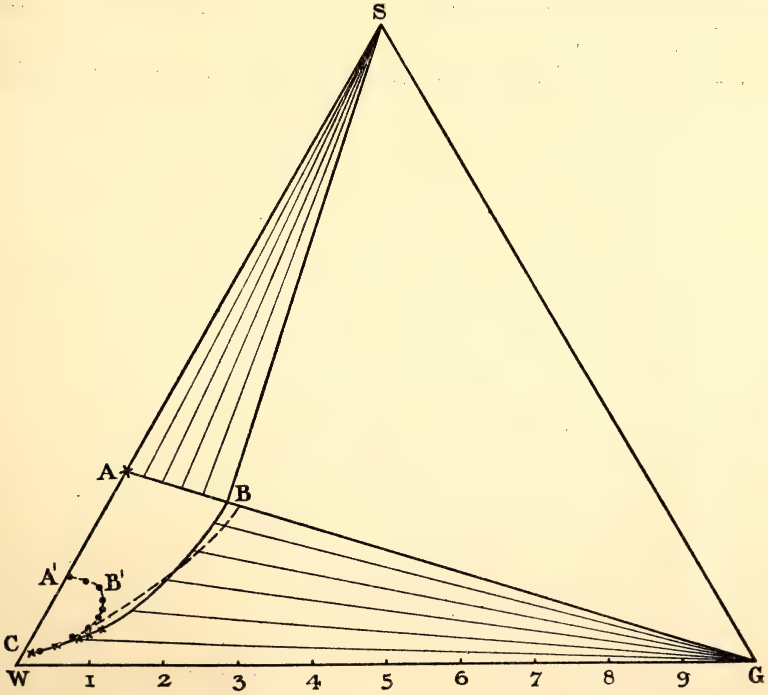

Fig. 1.

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* 'American Journal of Physiology,' vol. 14, 1905.

† *Loc. cit.*

Harris.* S is pure salt, W pure water, G pure globulin (edestin). The points on the curves, fixed by actual experiment, are shown in the usual way by dots or crosses. A′B′C is the curve for Na_2SO_4, ABC the curve for NaCl. The broken line extending to just beyond B is the probable form of the curve for H_2SO_4. The diagram is drawn as an isotherm for NaCl, water, edestin, the areas for the other salts being left out. The context is not very clear on the point, but the temperature of observation seems to have been 20°.

Each curve clearly consists of two distinct parts, AB, which is in equilibrium with solid salt, and BC in equilibrium with globulin crystals. The whole surface, therefore, consists of certain areas:—ABC, all points within which represent a homogeneous state, namely, a fluid solution of globulin, salt, and water; ASB, which is an area of heterogeneous states. Each point in it represents a mixture which cannot exist as a uniform state, but divides into two phases, one a fluid solution on the curve AB, the other a solid which is pure salt. WBG, again, is an area of mixture which cannot form a uniform state, but which divides into a fluid solution on CB, and into globulin crystals. SBG, each point within which represents a mixture which divides into a fluid having the composition of B and solid salt and solid globulin.

In fig. 2, portions of three isotherms for NaCl, water, serum globulin are

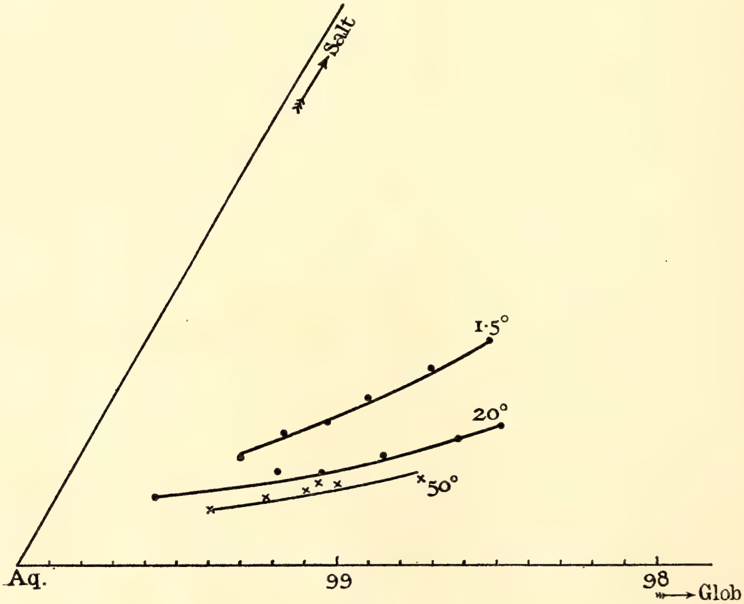

Fig. 2.

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* *Loc. cit.*

plotted to scale, the data being derived from Mellanby's paper. They cover only a very small fraction of the whole curve, ABC, but they are sufficient to show, (1) that the curve encloses an area which is placed on the line WS, as in the preceding case. This follows from the form of the curve, and from the fact that if sufficient salt be added, a point is reached on the line WS where salt solution is in equilibrium with solid globulin. (2) That the area enclosed increases with a rise of temperature.

This last fact, together with some observations of my own, enable us to approximate roughly to an isotherm for edestin at, say, 30°. When edestin is present in sufficient quantity, on warming a further amount passes into solution, so that we have a series of solutions in equilibrium with crystals. Beyond a certain temperature the crystals fuse, and we then have two fluid layers in equilibrium, the lower layer (as I have found by actual analysis) containing all three components.

Fig. 3 is a diagram of an isotherm at a higher temperature; it shows the area ABC enlarged, and it shows a new area, ROP, due to the appearance of a second series of solutions (the melted crystals). Of the shape of this area we know nothing. The curve ABC now contains three parts, AD in equilibrium with S, DE with G, and EF with the solutions which lie on OR.

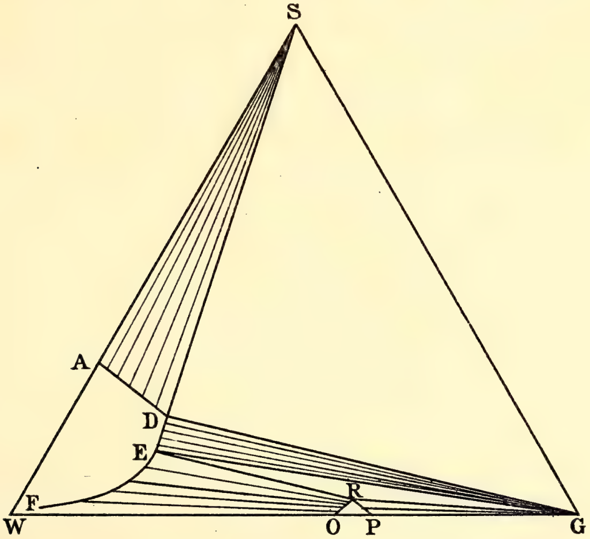
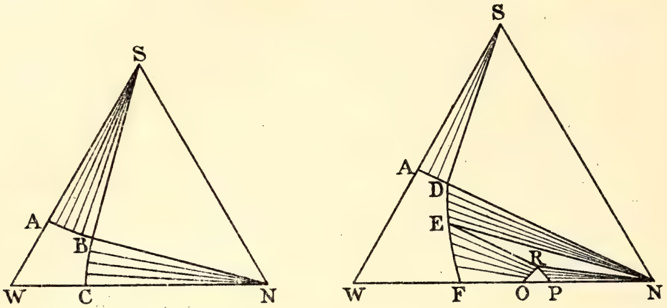


Fig. 3.

Fig. 1 does not represent the conditions for blood globulin at any temperature, since, in presence of water alone, a solid solution of water in globulin is formed. The tie lines from the curve BC, therefore, instead of focussing at G, end on a curve of unknown slope, which starts from about the middle of the line WG. In other words, the isotherms of blood globulins, over the region where we have any information to guide us, are of the form shown in fig. 4.



A B

Fig. 4.

This is as far as the known facts carry us. It now remains to compare the system with a similar one. Such a one is found in water, sodium chloride, and succinic nitrile, which has been studied by Schreinemakers.* In both there is a pair of immiscible bodies, salt, and globulin, or salt and succinic nitrile, each of which is partially miscible with the third substance, water. Fig. 4, A and B are two isotherms reproduced from Schreinemakers' paper. It is obvious that, in all essential features, they resemble the isotherms for globulin-salt-water already given.

It is clear, from this comparison and from the low viscosity, that globulin-salt-water is a system showing few abnormal features. It manifests decisive points of equilibrium, which resemble those in a comparable and purely crystalloid system. The other two globulin systems are as decisively abnormal, especially in the high viscosity, and the absence of definite transition points, and it is significant that, regarded from the purely physical standpoint, the essential difference should be the presence in the latter of large molecular aggregates, each carrying an electric double layer.

The presence of internal electrified surfaces adequately accounts for the high degree of inertia which obscures the transition points. What is the source of the electrification? In all cases in which it can be traced, the charge is due to molecular interaction of the type classed as chemical, and of that particular chemical type associated with the decomposition of neutral electricity, to which the name "ionisation" has been given. Burton† clearly shows that metals form hydrosols because they react with the solvent to form hydrides or hydroxides. Acid globulin, alkaline globulin, and soaps are salts.

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* 'Zeits. f. Physik. Chem.,' vol. 23, p. 417, 1897.

† 'Phil. Mag.,' April, 1906.

which form colloidal solutions because one of the radicles is of large size, is almost or quite immiscible with the solvent, and therefore readily forms molecular complexes which have the electric sign proper to the radicle when dissociated from its fellow in the salt molecule.

Each colloid particle undoubtedly carries on its surface a charge, since, without exception, they move in a uniform electric field. But in the system formed by each particle there cannot be any free electricity, any resultant charge. If there were, the system would, by Earnshaw's theorem, be unstable, and settling would occur the more rapidly the greater the charge. Experiment shows the opposite to be the case. The charge on each particle, therefore, must be bound by an equal and opposite charge on the liquid face opposed to it. The condition of double electric layers is therefore realised.

It has been shown by Waymouth Reid* that in a solution of proteid no osmotic pressure can be traced to the proteid, and I find that when a solution of globulin is dialysed against water until equilibrium is reached, the dialysee and dialysate have exactly the same electric conductivity. These facts, added to others already stated, afford overwhelming evidence that in these solutions the chief portion of the proteid is, in effect, removed from solution by being gathered into masses of more than molecular dimensions which are separated from the rest of the solvent by a surface. The masses of probably hydrated proteid thus form an internal phase. In colloidal salts such as soaps, solutions of globulin in dilute acid or alkali, solutions of caseinogen or of acid or alkali albumen, in which the internal phase is bounded by double electric layers formed by ionic interaction with the external phase, the stability of the system with reference to forces such as gravity will depend, in part, upon the potential difference between the two faces round each particle. The electric double layers will contribute to stability, since any movement of a particle through the fluid will develop free electricity in quantity proportional to the potential difference between the layers.†

Salts in small amount diminish the stability of these colloidal systems and bring about concentration; and salts, as has been shown experimentally by Wiedeman, Perrin,‡ Burton,§ and others, diminish the potential difference in the condenser system round each particle.

The contribution which the electrification of the internal surfaces makes to mechanical stability is seen in the high viscosity of these solutions as compared with solutions of globulin by salts which resemble them in containing large molecules, but differ in the total absence of electric layers from these molecules.

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* 'Journ. of Physiology,' vol. 31, p. 438, 1904.

† Helmholtz, 'Wied. Ann.,' vol. 7, p. 337, 1879.

‡ 'Journ. de Chim. Physique,' vol. 2, p. 61, 1904; vol. 3, p. 50, 1905.

§ 'Phil. Mag.,' November, 1906.

Solutions of globulins in salts introduce us to an entirely different aspect of colloidal solution. Here, again, the proteid contributes nothing to the osmotic pressure,* and, therefore, must be withdrawn from true molecular solution; but as all ionic interaction is suppressed by the large excess of salt present, there are no double electric layers formed, and in their absence viscosity is low and stable equilibrium conditions are rapidly reached.

The origin of the mechanical stability of this system, the nature of the influence which maintains the even dispersion of the proteid throughout the fluid, is difficult to explain, since any factor to which we can appeal seems also to involve the exertion of a finite osmotic pressure by the proteid.

If the distribution of the energy be considered, we arrive at the following:—Let the state of thorough mixing of the components be established, and let a movement under the influence of any mechanical force take place, so that the proteid becomes more concentrated in some region. In this region, owing to the concentration of the colloidal salt, the degree of hydrolysis and of ionisation will be diminished, and the available energy thereby increased.

The equation of hydrolysis which Arrhenius† gives shows that hydrolysis and ionisation diminish faster than concentration increases; therefore, with unequal distribution of the proteid the gain in available energy $(E - \tau\phi)$ will be greater in the regions of higher concentration than the loss in the regions of diminished concentration. The system as a whole, therefore, would gain in available energy, and the change from uniform distribution to unequal distribution could not take place unless work were done on the system.

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* Waymouth Reid, *loc. cit.*

† 'Zeits. f. Physik. Chem.,' vol. 5, p. 16, 1890.

Fatty Degeneration of the Blood.

By S. G. Shattock and L. S. Dudgeon.

(Communicated by Professor J. Rose Bradford, F.R.S. Received March 18,—Read April 18, 1907.)

Erroneous as histological research has proved Hunter's view of the organisation of effused blood to be, yet Hunter was the first to discern the biological truth that the blood is alive—alive in as strict a sense as is any other component part of the living body.

To view the blood as a tissue is a natural extension of the same conception, a tissue, that is, of mobile elements, and one in which the plasma represents a fluid, in place of a solid, intercellular material. Yet even in regard to this quality we may discover gradations between the opposite extremes like those presented by blood and cartilage, in such intermediate forms as mucous tissue, where the intercellular substance may be equally well called semi-fluid or semi-solid.

If there is a paradox in such an application of words, it is not so much that the incongruity marks an error of conception as that terms used in one stage of science become inadequate to compass biological truth, which as it grows under observation defies the artificial limitations which language would impose upon it.

In a similar manner has the common conception of a crystal been dissipated by the sheer force of observation. For the researches of Lehmann and of Schenck* have made it clear that certain fluids may retain the characteristic crystalline feature of containing doubly refractive bodies. The conception of a fluid crystal is not less paradoxical than that of a fluid tissue.

One peculiarity in the constitution of the blood whereby it differs from other tissues is, of course, the fact that although its cells, as in the more stable forms, undergo division, both mitotic and amitotic, and have in this sense a life of their own, yet the primary sources of its elements are the blood-forming organs—the bone-marrow, the hæmolymph and lymphatic glands, or other lymphatic tissue, and the spleen.

In considering diseases of the blood, therefore, there is a double question involved: there are firstly the changes in the blood arising from disease of the blood-forming organs, and, secondly, the abnormal changes of the formed blood, arising from lesions proper to the blood itself.

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* 'Flüssige Kristalle,' Leipzig, 1905; cited by J. C. Adami, F.R.S., and L. Aschoff, Roy. Soc. Proc.,' B, vol. 78, 1906.

Conceiving of the blood, then, as a complex tissue, and subject like any other to parasitic and bacterial infection, or partaking in the morbid changes of many other specific diseases, it might be imagined that it would likewise be subject to degenerations, and more particularly to the chief and most widespread of all of these, viz., fatty.

The blood-degeneration which has been as yet most studied (other than the so-called polychromatophilic of the red cells) is that usually regarded as glycogenic, and consists in the abnormal, *i.e.*, excessive, appearance of glycogen, chiefly in the finely granular polymorphonuclear leucocytes, although the granules occur also in the eosinophile or coarsely granular polymorphonuclear cells, and to a lesser degree in the lymphocytes.

To this form of degeneration (which is known also as iodinophilic or iodophilic, since the grains and granules take a brown colour after treatment with iodine solution) we will refer again in connection with certain ambiguous appearances met with by ourselves in blood-films treated with Scharlach solution.

If we except pathological effusions, such as those of pleurisy and peritonitis and purulent exudations, the process of fatty degeneration has hitherto been looked for and studied only in the solid tissues. Yet it will be apparent on theoretical grounds that in cases where fatty degeneration of the last-named tissues is due to a deficient oxygen-carrying power of the blood, or to a toxic condition of it, the state of the blood might react upon itself so as to induce similar changes in its own cells.

The chief causes of fatty degeneration in general, excluding those of a local character which produce only a local change and have no interest in connection with the present subject, are profound anæmias and certain forms of toxæmia, whether bacterial or of inorganic causation, as especially illustrated in phosphorus poisoning.

In the first the fatty degeneration of the various organs results from the reduction in the oxygen-carrying power of the blood. In the second, it results directly from the injurious action of the toxin upon the cells of the tissue.

Our inquiry, therefore, is: Does the blood itself suffer similarly in these two groups of morbid conditions?

(1) Do the leucocytes (leaving the red cells out of consideration) suffer from a defective oxygen-carrying power of the blood itself?

(2) Do the leucocytes suffer from the direct action of toxins circulating in the blood in profound toxæmia?

In the blood, as in other tissues, the distinction must be drawn between fatty infiltration and fatty degeneration.

Without entering into the chemical question as to the source of the fat in fatty degeneration, how far, *i.e.*, it is due to a transformation of the proteid of the cell (as once held), and how far to the accumulation of fat reaching the cell from without and appearing in it because not utilised, the cardinal difference between the two processes remains, even though the source of the fat should be the same in both.

In the one case the fat accumulates or is stored in a cell of which the cytoplasm is uninjured; in the other it appears for the reason that the latter is damaged and rendered incapable of utilising the fat which reaches it.

With the fat that is not free, but combined, in the cell, neither term deals; the presence of this is not recognisable, either by its microphysical or microchemical properties.

Technique.

At the outset it is obviously essential to exclude the use of fat solvents, like absolute alcohol and ether, as fixing reagents, and essential also that the blood should not be dried. One preliminary method we tried was the use of blood clot, but it proved less satisfactory than that of making films.

Microscopic sections are readily cut from cylinders of blood that has been received into, and allowed to clot in, narrow tubes, the clot being afterwards fixed in salt-formol followed by 80-per-cent. alcohol. Such sections can be stained for fat like those of other tissues. The method, again, of using washed corpuscles (*i.e.*, corpuscles washed in salt solution after having been separated by the centrifuge from blood in which clotting has been prevented by the addition of citrate of sodium) offers no special advantages, and after making a trial of it we did not adopt it.

The method finally chosen was that of making films on slides and cover glasses. The film from first to last must be kept moist, and treated, in short, throughout as a section of any tissue which is to be studied for the presence of fat. The reason for this will be obvious, for any fat in the leucocytes would, in the dried film, tend to transude, and become lost by diffusion in the surrounding area.

The film having been made on the slide in the usual manner, by drawing a second slide in front of the blood, the slide is immediately placed with the film downwards in a specially devised chamber of formol vapour.

The chamber we use is a deep, oblong glass with a ground edge, and is itself hardly larger in sectional area than an ordinary microscopic slide. This is packed nearly to the top with cotton wool thoroughly soaked with formol (40 per cent.), and on the top of the wet wool is laid a grating of perforated zinc, near either end of which is soldered a cross strip of zinc wire; the latter prevents the film from touching the grating, through which the vapour freely passes to fix the film. The chamber is covered with a piece of overlapping plate glass, after vaseline has been painted over the edge.

Before the slide is prepared, cover-glass films can be made in the usual manner, and placed with the blood upwards upon the perforated zinc. The glass lid being immediately replaced, the slide film is prepared and introduced with the film downwards over the cover glasses, from off which it is kept by means of the cross pieces of zinc wire on which it rests.

After not less than 15 minutes (though the films may be left in the vapour for 24 hours or longer, if convenient) the slide is removed and at once placed in the vertical position in a glass of Scharlach for 24 or 48 hours. The cover glasses are placed separately in hollowed glass blocks of the dye, accurately covered to prevent evaporation.

After a certain amount of experience we adopted, as a check, the practice of treating one of the cover glasses after removal from the formol, and before its transference to the Scharlach, with absolute alcohol, followed by ether and again by absolute alcohol; the object of this was to remove any fat or fat-like bodies from the cells of the film. At first, in making the cover-glass films, we adopted the plan of adding salt solution to the blood on the cover, in order to prevent any possibility of the film drying; the film itself was made on each cover glass separately by means of a platinum loop. This precaution was found to be unnecessary, and we therefore discarded it.

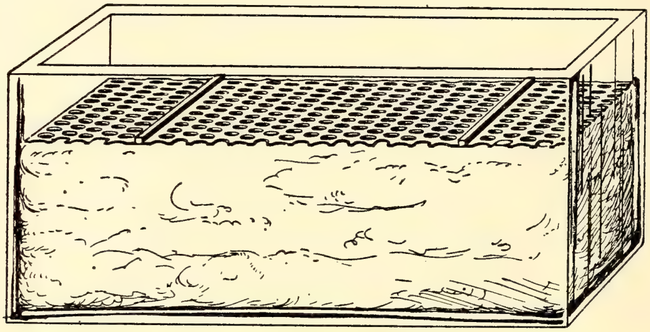

Fig. 1.—A diagram showing the glass formol chamber and zinc grating used to fix the blood films. The glass lid is not represented. Below the perforated zinc the space is filled with cotton wool soaked in formol solution, 40 per cent.

The use of alcoholic solution of Scharlach as a fat stain requires much care, for the dye readily precipitates. The solution we employed was made by saturating 75 per cent. absolute alcohol in the cold, and subsequent filtration through paper first wetted with alcohol. In the case of a slide, the latter was placed straight from the formol chamber into the Scharlach solution, and after 24 or 48 hours withdrawn, washed for a few seconds in 75-per-cent. alcohol, then in distilled water, and after this stained in a bath of hæmalum for three minutes; this was followed by washing in distilled water, and then in tap water, the film being finally protected with a long cover glass after the application of Farrant's medium. The cover glasses were prepared in a similar manner.

Our observations were commenced in April, 1906, and on May 29, 1906, at the meeting of the Pathological Society of London, held on that date,* we made a preliminary statement to the effect that we had discovered the existence of fatty degeneration in the blood in certain morbid conditions. We are not aware that any similar observation has been published before this announcement. The only work of a similar kind of which we know, indeed, is that carried out by Professor Antonio Cesaris-Demel, at the Institute of Pathological Anatomy of the University of Pisa. Of this a preliminary note was published very shortly after our own, viz., in June, 1906, having been communicated to the Royal Academy of Medicine of Turin at its sitting of June 8, 1906.† The title of the note is, "On Degenerative Changes of the Leucocytes of the Blood, studied by the Method of Coloration in the Fresh State." The author gives no detailed account and no figures, although he states that the stain used by him was Sudan III, one closely allied to that used by ourselves.

Our examination of blood films extends altogether to 79 cases and comprises various morbid conditions. Nevertheless, it is in comparatively few that we have satisfied ourselves of the presence of fat in the leucocytes, *i.e.*, of points or droplets presenting the same physical characters and coloration with Scharlach as those seen, *e.g.*, in the typical form of fatty degeneration of the cardiac muscle or renal epithelium.

List of Diseases in which the Blood was examined for Fatty Degeneration.

Pernicious anæmia.
Toxæmia of pregnancy.
Subphrenic abscess.
Chlorosis.
Diphtheria.
Appendicitis.
Lymphadenoma.
Acute meningitis.
Myelæmia.
Influenza.
Exophthalmic goitre.
Acute pneumonia.
Chronic Bright's disease.
Purpura.
Pericarditis (rheumatic).
Anæmia (secondary).

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* 'Brit. Med. Journ.,' June 2, 1906.

† 'Giornale della R. Accademia di Medecina di Torino,' Giugno-Luglio, 1906.

Post nephric abscess.
Anæmia of undeterminal origin.
Pleurisy and pericarditis.
Septic bronchopneumonia.
Carcinoma of pylorus.
Sub-acute rheumatism, probably gonorrhœal.
Septic nephritis.
Myxœdema.
Hepatic suppuration.
Ulcerative endocarditis.
Rickets.
Acute cerebro-spinal meningitis.
Diabetes and lipæmia.

In the case of some of these diseases more than one example was studied.

List of Diseases in which Fat was found in the Leucocytes.

Chlorosis.
Chronic Bright's disease and anæmia of chlorotic type.
Carcinoma of pylorus and anæmia of chlorotic type.
Influenza.
Pleurisy and pericarditis.
Toxæmia of pregnancy.
Acute pneumonia.
Purpura.
Diabetes and lipæmia.
Myelæmia.
Lymphadenoma.
Acute cerebro-spinal meningitis.

If these cases are analysed it will be found that most of them come in the category of toxic diseases, some of an acute kind, others of a chronic. Of the three cases of Myelæmia in which fatty degeneration of the finely granular leucocytes was found, one was acute, two were chronic.

The degree of fever present in these toxæmic diseases varied according as the condition was one of intoxication or infection. In one of the most pronounced examples of fatty change, viz., the toxæmia of pregnancy, the pyrexia was very slight.

In the toxic series may be placed:—

Toxæmia of pregnancy.
Acute pneumonia.
Myelæmia.
Purpura.
Acute cerebro-spinal meningitis.
Influenza.
Pleurisy and pericarditis.
Lymphadenoma.
Chronic Bright's disease (associated with some degree of chlorotic anæmia).

In regard to this group of cases we believe that the fatty change present in the finely granular polymorphonuclear leucocytes results from the direct action of the toxic substances present in the blood itself; that is to say, that the change is one of proper fatty degeneration as distinguished from the ingestion or storage of fat by a healthy cell.

The fineness of the fatty points, their varying number in different cells in the same film, and the fact that they occur in abundance in cases where the condition of the patient excludes the possibility of so large an amount of fat being merely ingested from the blood by the circulating leucocytes, all point to this conclusion. Thus in the three cases of acute pneumonia the blood was taken, in one, on the sixth day, two hours after the crisis; in another, on the fifth or sixth day, before the crisis; and in the third, five hours after the crisis. Furthermore, the fact that in such an acute disease fatty degeneration actually does occur in the renal epithelium and cardiac muscle proves the ability of the toxins to effect such damage also upon the circulating leucocytes.

That the degeneration of the leucocytes has occurred in the blood and not in the bone-marrow may be held as almost certain, since had so marked a change taken place whilst the cells were still in the marrow, it is unlikely that they would have entered the vessels; they would have remained *in situ*. Upon the actual condition of the bone-marrow we have, with one exception, no observations.

When, as in myelæmia, the fat occurs likewise in myelocytes in the blood, we should think, similarly, that the change occurred in the cells after they had reached the blood-stream.

The leucocytes most degenerated probably die, and might by their disintegration furnish a certain amount of free fat in the plasma.

Not that this is the sole source of the free fat in the blood which was present in some of the cases, for its amount is too great for such an explanation. Most of the free fat must be regarded as derived from the intestine, or as transferred from the common connective tissue to the blood plasma for the use of the more important organs. The fat in some of the leucocytes under these circumstances is doubtless ingested, but the fact that in some of the cases where the fat in the leucocytes was well pronounced no free fat was present in the plasma shows that this cannot be advanced as the sole explanation.

The presence of fat in some of the myelocytes of the blood film in myelæmia, again, cannot be due to ingestion, since these cells have no phagocytic powers. It might have been thought, *a priori*, that the presence of fat in the finely granular polymorphonuclear leucocytes would be a sign of grave significance as indicating not only a high degree of toxæmia but an interference with the function of cells, the phagocytic integrity of which is of such importance in the cure of a disease like pneumonia. For the leucocytes so altered must have lost for the time their physiological capacities. They would be much in the same condition as the pus cells in an acute abscess, rendered fatty by the toxic action of pyogenic micro-organisms. Indeed, a study of cover-glass films made from the pus of an acute abscess and treated precisely as those of blood reveals the presence of scarlet points and droplets within a large number of the finely granular polymorphonuclear leucocytes, which are exactly matched by those in the leucocytes of certain of the blood films described. In some of the pus cells (as in the blood) the red points are quite minute (as they were in the case of influenzal pleurisy); in others larger (as in one of the cases of acute pneumonia); or still more so, as they were in the case of pyloric carcinoma associated with chlorotic anæmia.

It is noteworthy, too, that in the pus cells so affected the nuclei may exhibit no fragmentation. Nor have we ever seen nuclear fragmentation in the fatty leucocytes in blood films.

This prognostic conjecture, nevertheless, is not borne out by observation.

Thus in the three cases of acute pneumonia in which a certain number of the polymorphonuclear leucocytes contained fat, recovery ensued. In one case the blood was examined on the sixth day, two hours after the crisis; in another on the fifth or sixth day, before the crisis; and in another five hours after the crisis.

The actual number of leucocytes showing the fatty change in these three cases, however, was not excessive, and although the phagocytic power of such cells would, doubtless, be diminished or destroyed, the great excess of leucocytes present would compensate for such a loss. Within two or three days after the crisis the leucocytosis has disappeared; the leucocyte count is practically normal.

This rapid disappearance of the leucocytic excess from the blood is largely due to the vast immigration of leucocytes into the infected organs, the lung more especially; the surplus yet remaining would disappear, as having no further purpose to serve. For all observation goes to show that the rapid amelioration following the crisis is due to the activity of the finely granular leucocytes.

MacDonald* has shown that the opsonic index attains its maximum at the crisis. And Rosenow† concludes from his work that pneumonia serum has no pneumococcidal effect; that the opsonin during and shortly after crisis (in cases that recover) seems to rise above the normal, and that the pneumococcidal action of pneumonic blood is the result of the combined action of serum and leucocytes—phagocytosis and intraphagocytic digestion. It must not be forgotten, moreover, as a general consideration, that the degenerating cells in the blood may recover, like those, *e.g.*, of the kidney in toxic diseases, in which the damage done to the renal epithelium is shown by the temporary presence of albumen and casts in the urine.

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* 'Path. Soc. Trans.,' London, vol. 57, p. 45, 1906.

† 'Journal of Infectious Diseases,' vol. 3, June 30, 1906, Chicago.

In all the cases we have examined the fatty changes were confined to the finely granular polymorphonuclear leucocytes, that is, to the cells which take the active part in phagocytosis ; the most active cells, as being the more highly differentiated, are those most affected by the toxin.

We have never seen any such change in the lymphocytes—a class of cell devoid of phagocytic power.

The inactivity of the lymphocytes is a striking comment upon the fanciful views once held, that the lymphatic tissue, whether of the tonsils, lymphatic glands, or intestinal track, constituted a highly important line of defence against microbial invasion. As a matter of observation it is in the solitary and Peyerian glands of the intestine that tubercular infection takes place ; and once a tubercular process has commenced in a member of a lymphatic chain it spreads from gland to gland until the whole are involved.

Anæmia.

In this group of diseases the following of the cases in which fat was found in the leucocytes may be placed :—

(1) Chlorosis.

(2) Carcinoma of the pylorus, associated with anæmia of the chlorotic type.

In chlorosis the fatty changes occurring in the finely granular polymorphonuclear leucocytes are attributable to the deficient oxygen-carrying power of the blood. No direct observations upon the state of the organs appear to have been made in this disease, although it is commonly assumed that they are affected with fatty degeneration ; the dilatation of the heart observed clinically being especially so explained. Observations upon the blood-forming organs are equally wanting.

In chlorosis the most marked change in the blood is the excess of water—hydræmic plethora. The number of red cells in a given volume is diminished ; the absolute number is increased ; and similarly, whilst the percentage of hæmoglobin may be reduced to 40 or even to 30, the total hæmoglobin content remains normal, and the oxygen-capacity undiminished.

As the reduction in the oxygen-carrying power affects the tissues supplied by the blood, so it may affect the nutrition of the leucocytes in the blood itself.

In the particular case of chlorosis in which fat occurred in the leucocytes the blood was taken at 2 o'clock p.m., the patient having had no food since breakfast. The plasma, as studied in the blood films, contained free fat, the presence of the latter, under the circumstances mentioned, being possibly due to its less ready manipulation in consequence of the excessive bulk of water in the blood.

The amount of fat in the finely granular leucocytes in this case is very striking; every cell held a certain number of fine scarlet points or droplets, and many are loaded. How much of the fat in the leucocytes is to be ascribed to ingestion, and how much to degeneration, it is impossible to say.

As in the case where œdema follows venous obstruction the transudation occurs only after the stasis has led to damage of the endothelium, so in a somewhat similar way, in the case of anæmia, we should regard the deficient oxygenation as involving injury to the leucocytes; the intracellular fat appears in consequence.

In the case of pyloric carcinoma there was an associated chlorotic anæmia arising from the ill-nourished condition of the body in general, including the blood-forming organs.

The fat droplets in the leucocytes, of which only a certain number were affected, are of conspicuous size, the change being a far advanced one. No free fat was present in the plasma.

And finally, in the case of diabetes and lipæmia, in which typhoid infection terminated life, the amount of free fat in the plasma makes it clear that much of the fat in the leucocytes must be ascribed to phagocytic ingestion, but how much to this, and how much to the toxic condition of the blood, cannot be stated. The peritoneal fluid removed after death was full of fat droplets. The bone-marrow showed a highly abnormal number of large endothelial cells holding an abundance of multiple fat droplets. The gall-bladder held no bile, and its contents were so white that they were at first thought to be purulent; microscopic examination revealed the presence of free fat and shed columnar cells.

It is interesting to note, in passing, that the bases of these cells were loaded with fat, which had evidently been ingested by them whilst *in situ*, as it is by those of the intestine. The absorptive capacity of the epithelium of the gall-bladder has been shown, also, by Aschoff.

The Presence of Granules in the Leucocytes, which Stain of a Deep Brown Colour.—Scharlach Granulation.

From the foregoing list of diseases in which fat was found in the finely granular polymorphonuclear leucocytes, some, in which a fatty degeneration might have been particularly anticipated, are conspicuous by their absence.

Two such would certainly be diphtheria and pernicious anæmia. In both these diseases, however, we have found the leucocytes laden with fine granules, which, though deeply stained, do not exhibit the transparency and proper red colour of ordinary fat.

We may first give a list of the cases in which these deep brown granules occurred; the phenomenon itself we may call, for convenience, Scharlach granulation:—

Subphrenic abscess.
Exophthalmic goitre.
Pneumonia and empyema.
Pernicious anæmia.
Diphtheria (6 cases).
Appendicitis.
Pericarditis (rheumatic).
Acute meningitis.

In discussing the nature of these granules, various possibilities require consideration. That the granulation is not a mere precipitate of Scharlach within the cytoplasm of cells that have been imperfectly fixed is shown by the fact that the diphtheria blood films (in which, amongst others, it occurred) were fixed in formol vapour for over 24 hours.

It is noteworthy that the normal granules of the eosinophile leucocytes exhibit a somewhat similar coloration, though of a less intense degree. Against the identity of the granulation with the substance composing the eosinophile grains, however, must be placed the circumstance that the latter are equally well coloured in both pathological and normal blood films after the films have been treated with absolute alcohol; the fine granules under discussion, on the contrary, are not demonstrable by means of Scharlach if the films are first treated with absolute alcohol.

The degree of Scharlach granulation, moreover, present in some cases (every finely granular leucocyte being laden) excludes, we think, the explanation that the granules in question are eosinophile, which have been discharged from coarsely granular cells, disintegrated, and subsequently ingested by the finely granular leucocytes.

In this connection it may be further mentioned that no eosinophilia has been present in any of the cases as a possible source of such an excessive amount of Scharlach-staining substance.

The Scharlach granulation is not due to the coloration of the proper granules of the cell; for in some of the leucocytes comparatively few granules occur, and in these cells the proper granules are recognisable, though unstained.

The granulation is not unlike the glycogenic, as displayed in the leucocytes after treatment of blood films with iodine solution. In most cases the iodine reaction takes the form of a diffuse brown coloration of the cytoplasm of the finely granular polymorphonuclear leucocytes, but it may take that of a fine stippling. A still further likeness is to be found in the occasional presence of similar iodophilic granules in the plasma; for fine extra-cellular granules may occur also in the Scharlach preparations.

Although the iodophilic reaction may be seen in normal blood, in pronounced degree it occurs only under pathological conditions, and observers are agreed in viewing it as evidence of cell degeneration, brought about in most cases by a toxic cause.

We exclude this explanation, however, for the reason that the Scharlach granulation is not to be observed in control cover-glass films if treated with absolute alcohol before being transferred to the dye. The glycogenic reaction, on the contrary, is as readily obtained after this treatment as without it.*

We have, moreover, found that glycogen is uncoloured by Scharlach. If fat-free glycogen (from the rabbit's liver) is dissolved in distilled water and precipitated by alcohol, the wet sediment, when treated with Scharlach for 48 hours and examined microscopically, is seen to consist of fine spherical granules which are quite untouched by the dye. The same sediment stains of a mahogany brown when submitted (after drying on an albuminised cover glass) to the action of iodine vapour for 20 minutes, and afterwards mounted in Ehrlich's iodine medium.

The absence of the Scharlach granulation when the blood films have first been treated with absolute alcohol excludes the possibility of the granules being blood pigment which has been ingested by the leucocytes.

We have found the Scharlach granulation in some of the finely granular polymorphonuclear leucocytes in cover-glass films made from the splenic pulp in a case of acute peritonitis, the films having been fixed in formol and treated precisely like those of the blood. In this observation, also, the exclusion of blood pigment is brought out by the fact that the deep brown granules are not to be seen in the films stained by Leishman's method.

Does the Scharlach granulation represent a substance in which fat is not free, but combined? Is it allied to lecithin or myelin, both of which are known to occur in many kinds of cell? Lecithin stained from egg-yolk is not coloured with Scharlach.†

In sections of an enlarged prostate which we have examined, although the presence of fat was demonstrated by Scharlach treatment in some of the epithelial cells, the free granules in the acini (generally held to be lecithin) were quite uncoloured.

By the same want of colourability, after treatment with formol, myelin may be excluded. In medullated nerve fixed in formol and afterwards teased and immersed in Scharlach solution for 24 hours, the myelin of the individual fibres exhibits practically no coloration; it is equally unaffected by osmic acid, if the nerve is first fixed in formol.

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* J. Barnicot, 'Journal of Pathology,' vol. 11, p. 304.

† T. R. Elliott and I. Tuckett, 'Journal of Physiology,' vol. 34, p. 350.

As regards the coloration of human fats, that is, of their fatty acids which are the proper colourable substances, pure oleic acid is stained of the typical red colour with 75-per-cent. alcoholic solution of Scharlach.

From human liver Leathes has extracted another fatty acid, the colourability of which we have been able to test upon cover-glass smears. The smear, after being treated with formol vapour, stained with Scharlach, and finally washed in distilled water and mounted in Farrant's medium, is transparent, and of an orange or orange-brown colour; it presents nothing suggestive of the Scharlach granulation.

From human heart-muscle Leathes has also extracted a fatty acid other than oleic, and not identical with that referred to from the liver.

The fact, however, that in fatty degeneration of this muscle, as studied in diphtheria, the fat that appears is from the first stained of a brilliant red by Scharlach shows that, whatever fat besides olein may be present, it is not the substance constituting the Scharlach granulation.

There is, to conclude, the possibility that the granulation is due to a soap, the colloidal nature of which prevents its diffusion into the substance of the cell.

Various fermentative capabilities have been ascribed to the leucocytes. They are certainly proteolytic; and both amylolytic and lipolytic powers have been attributed to them.

It is conceivable that the fat droplets, on their first appearance in the leucocytes, might be split, with the resulting formation of an intracellular soap arising from the combination of fatty acid with calcium, sodium, or potassium.

Using pure oleate of calcium (the only soap of the three not soluble in water), we find that cover-glass smears, after being treated as the blood films, fail to exhibit the dark brown colour and opacity of the Scharlach granulation; the particles take a pale brown or orange tint.

For the present, therefore, we are forced to leave the nature of the Scharlach granulation undetermined. Nevertheless, its appearance in diseases in which a fatty degeneration of the leucocytes might have been anticipated, and the fact that in some cases of acute pneumonia the proper Scharlach reaction of the points in the cells is obtained, whilst in others it is the brown opaque Scharlach granulation, makes us conclude that the granulation is indicative of a degeneration; and seeing that it is not obtainable after the treatment of the blood films with absolute alcohol, we conclude that it is allied to that known as fatty.

In conclusion, we desire to express our thanks to Dr. G. Buckmaster, and to Dr. J. B. Leathes, for criticism and help in regard to certain of the points raised by the investigation.

Some Points in the Development of Ophiothrix fragilis.

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(Received May 6,—Read June 13, 1907.)

The subject of which the present communication is a brief preliminary account has occupied my attention during the last five years, and a full account will be published shortly. Meanwhile, a brief outline of the main results is given here.

Ophiothrix fragilis is one of the commonest species of British Ophiuroidea. Especially in the neighbourhood of Plymouth, where the material for this research was obtained, this species is found in immense swarms, so that, on certain kinds of bottom, hundreds of individuals are brought up with each haul of the dredge. During the months of June and July it spawns, and on some occasions the Plankton captured above the beds of gravelly sand on which it lives is full of its larvæ in all stages of development.

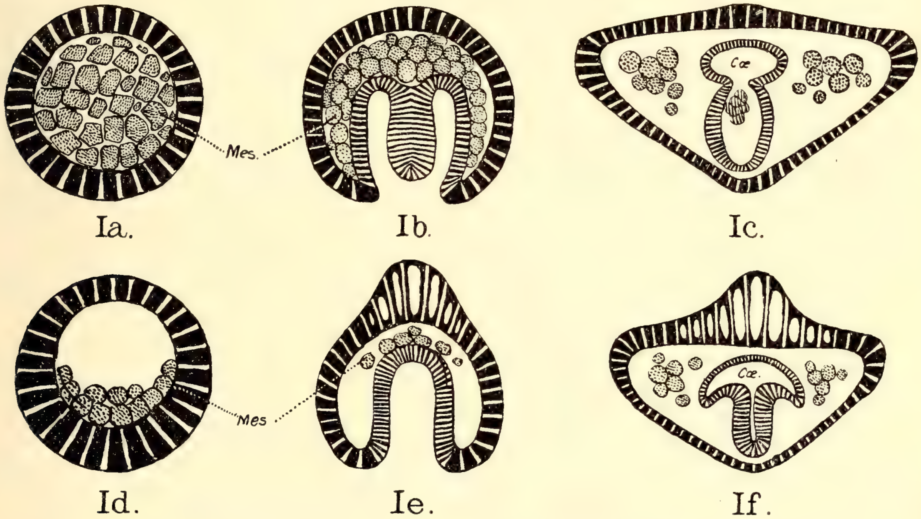
A large quantity of this Plankton, captured during July, 1899, formed the principal part of the material, but this was supplemented by artificial fertilisation carried out in the laboratory of the Marine Biological Association in the years 1898, 1899, and 1905.

The eggs are small (about 0·1 mm. in diameter) and opaque owing to the possession of a reddish-brown yolk. The development was observed to be completed in 28 days, but the rate of development depends on the amount of food supply in the water, and it is probable that in the sea the eggs develop more quickly than under the most favourable circumstances in the laboratory.

Only on one occasion (June, 1898) was it found possible to rear the larvæ through their development until metamorphosis was completed; this was due to the presence of an unusual amount of Phytoplankton in the water on that occasion, but on other occasions an abundant supply of the early stages of development was obtained, so that the gap in the series of larvæ captured in the Plankton was completely filled.

If artificial fertilisation is carried out in the usual way, viz., by dissecting out the ovaries and shaking them over a beaker of clean sea-water in order to let the eggs fall out, and then shaking in the water a piece of testis extracted from a ripe male, the development takes place in the manner which I have already described to the American Society of Zoologists (1), and which is illustrated in figs. 1*a*, 1*b*, and 1*c*. The egg segments so as to give rise to a solid mass of cells or morula.

Fig. 1.



Figs. 1*a*, *b*, *c*. Three stages in the abnormal development of *Ophiothrix fragilis*. *Mes*., mesenchyme. *Cœ*., cœlomic rudiment.

Figs. 1*d*, *e*, *f*. Three stages in the normal development of *Ophiothrix fragilis*.

When the embryo escapes from the egg-membranes and commences to lead a free-swimming existence it has an ellipsoidal form, and consists of an outer mantle of columnar ciliated cells and of an inner mass of rounded cells. It very much resembles the "planula" larva of a Cœlenterate. Subsequently an invagination takes place at one pole of the egg, and the archenteron is formed. But the invaginated cells do not form a single layer. A multiplication of cells takes place at the vegetable pole before the invagination takes place, and when this occurs it takes place in such a manner as to give rise to a sac with a tongue of cells projecting into the lumen. A similar development in the case of *Ophiura brevis* has been described by Grave (2).

The larva soon assumes a triangular form (fig. 1*c*), one angle being the posterior end of the animal, whilst the other two are the rudiments of the postero-lateral ciliated arms, which in this species attain an extraordinary length.

The interior cells of the morula are now seen to be the skeleton-forming cells of the calcareous spicules which support these arms. The cœlom arises as a vesicle from the apex of the archenteron, and shortly afterwards the stomodæum makes its appearance in the centre of the anterior surface. The tongue of cells projecting into the archenteron is gradually absorbed.

Until 1905 no doubt existed in my mind that the course of development above described was the normal one; but in that year, in addition to the ordinary methods of artificial fertilisation, the device was tried of placing males and females together in half-gallon jars filled with pure sea-water and allowing them to spawn naturally. A vigorous culture was obtained in this way, and the development differed in some important particulars from the type described above.

Segmentation results not in the formation of a morula, but of a thick-walled blastula (fig. 1*d*), one side of which becomes specially thickened and buds off mesenchyme cells. The embryo now escapes from the egg-membrane and becomes a larva. Soon at the pole from which the mesenchyme was produced a regular invagination takes place, with no trace of the tongue of cells described above.

Mesenchyme cells continue to be budded off from the apex of the growing archenteron. At the same time a conspicuous feature makes its appearance at the animal pole of the larva. This is a crest of vacuolated cells (fig. 1*e*), which has a triangular shape, and seems to be an apparatus for the floating of the larva, which lasts until the ciliated arms are developed.

The cœlom next makes its appearance as a thin-walled sac, greatly resembling in shape the cœlomic rudiment in the larva of *Asterina gibbosa* (fig. 1*f*), and at the same time the rudiments of the postero-lateral arms appear. The vacuolated crest diminishes slowly in size; when it first appears its height is as great as the entire length of the larva, but by the time the stomodæum appears it has disappeared. Previously to this the cœlomic rudiment has become separated from the gut as a single vesicle. This then divides into right and left halves, and the stomodæum joins the archenteron, which now shows beginning of the constrictions which mark it off into œsophagus, stomach, and intestine.

Pausing for a moment to reflect on the meaning of the two types of development described above, it is clear that the difference between them cannot be due to the fact that in the one case the genital organ was dissected out, and in the other case that the animal was allowed to spawn. In some cases larvæ with a high vacuolated crest were obtained from artificial fertilisation. It appears to be clear that we must assume that eggs are capable of fertilisation before they are quite ripe, and that the fact that eggs can be fertilised is no proof that they are fully ripe or that the resulting development is normal. This conclusion has, I think, a somewhat important bearing on the experimental studies for which the eggs of Echinodermata have furnished the material. Notably the statements which some authors have made about obtaining ripe eggs from sea-urchins like Strongylocentrotus all the year round must be received with great caution.

When engaged in the study of the development of *Echinus esculentus*, I noticed that if one attempted to fertilise the eggs of half-grown specimens, larvæ with imperfectly developed arms resulted, which only lived a few days.

The fact, however, that the abnormal development of *Ophiothrix fragilis* has a considerable resemblance to the normal development of *Ophiura brevis* is of interest, as showing how far reaching in its influence on the subsequent development is the condition of the egg at the moment of fertilisation, and the idea is suggested that here, perhaps, is to be found the origin of variations.

We left the normal development at the moment when the cœlom had been completely separated from the gut and had been divided into right and left halves. The ciliated band had been formed, and its first two outgrowths, the postero-lateral arms, had appeared. The stomodæum had joined the gut.

The madreporic pore is now formed by the union of an ectodermic ingrowth with a short outgrowth from the left cœlom. The left cœlom is now distinguishable from the right by its more distinct lumen—an indication of the fact that it gives rise to the so-called ampulla of the stone-canal. I have searched in vain for any trace of a right pore-canal such as has been found by Grave in the case of some ophiurid larvæ. From both cœloms, muscle fibres are developed which surround the œsophagus of the larva and endow it with the power of peristaltic motion. The other larval arms then appear, first the antero-lateral pair at the sides of the mouth and then the post-oral pair at the sides of the anus. The cœlom now undergoes transverse division on both sides into anterior and posterior cœloms, the division on the left side being completed before that on the right (fig. 2). The posterior cœlom on both sides is at first a solid nodule of cells which later acquires a lumen. Next, the last pair of ciliated arms, the postero-dorsal, appear, and then the anterior cœlom on both sides again prepares for division. As before, a nodule of cells buds out from the posterior end, but this time the division is not completed. On both sides the posterior portion, which will form the middle division of the cœlom, is not completely separated from the anterior division. On both sides it becomes hollow. On the left side it buds out five finger-like prolongations (fig. 4), thus proclaiming itself to be the rudiment of the hydrocœle, or water-vascular system of the adult. Sections demonstrate that its persisting connection with the anterior cœlom which constitutes the stone-canal, as in the case of *Asterina gibbosa,* is situated between lobes 1 and 2.

Fig. 2.

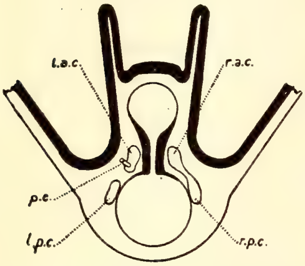


Fig. 3.

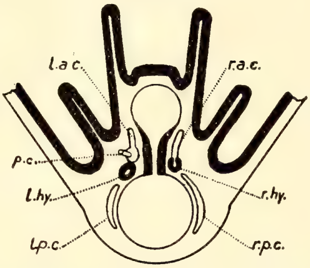


Fig. 4.

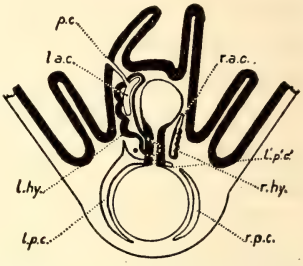


Fig. 2.—Diagram of larva, showing cœlom divided on left side and dividing on right side.

Fig. 3.—Diagram of larva, showing formation of rudiments of hydrocœles from the posterior ends of the anterior cœlomic sacs.

Fig. 4.—Diagram of larva, showing first stages of metamorphosis. Notice migration of pore towards the right. *l.a.c.*, left anterior cœlom; *l.p.c.*, left posterior cœlom; *l.hy.*, left hydrocœle; *l'.p'.c'.*, right horn of left posterior cœlom; *r.a.c.*, right anterior cœlom; *r.hy.*, right hydrocœle; *r.p.c.*, right posterior cœlom; *p.c.*, pore-canal.

The metamorphosis of the larva into the adult form now commences. This is initiated by a preponderant growth of the left side. The hydrocœle enlarges, its lobes grow longer, and its anterior end commences to extend dorsally to the stomodæum (fig. 4). Soon the left antero-lateral arm is involved and is pushed over to the right (fig. 4). This is the first sign of the metamorphosis visible to the naked eye. As metamorphosis proceeds, this left arm comes to lie close to its fellow of the right side, and the first lobe of the hydrocœle becomes completely dorsal to the œsophagus. The left posterior cœlom sends a horn over to the right side beneath the œsophagus, and the posterior end of the hydrocœle extends over to the right, parallel to this horn, eventually meeting the anterior end and converting the hydrocœle into the water-vascular ring.

The movement of the anterior end of the hydrocœle carries with it, as already indicated, all the neighbouring parts of the animal, so that the pore-canal and left anterior cœlom are carried over to the right and thus come into close proximity with the right hydrocœle, which occasionally, as is shown in fig. 4, *r.hy.*, undergoes a development similar to that of the left side, presenting five lobes. Often it presents two lobes, but normally it is a thin-walled vesicle without lobes.

In Asteroidea and Echinoidea, the right hydrocœle is from the beginning close to the dorsal pore, and on this account Masterman (3) and Goto (4) have called it the central cœlom and denied its homology with the left hydrocœle. In *Ophiothrix fragilis* there can be no question on the subject, for the right hydrocœle is, from the beginning, on the right side of the larva, and its dorsal position in the other groups may be explained by supposing that it is right with regard to the *adult* position of the mouth. As the *adult mouth* is on the left side of the larva, right with respect to this mouth will be nearly mid-dorsal with respect to the larval mouth. But in Ophiuroidea, where the larval mouth persists as the adult mouth, the right hydrocœle is on the right of it from the beginning, and the pore, originally situated on the left side, moves round so as to assume a mid-dorsal position.

Little remains to be added in this preliminary account. The arm rudiments are outgrowths of the left posterior cœlom and, counting from the dorsal end, arm rudiment No. 1 comes to support hydrocœle lobe No. 2, as in *Asterina gibbosa,* whilst arm rudiment No. 5 supports hydrocœle lobe No. 1. As in *Echinus esculentus,* inter-radial ridges alternating with the lobes of the hydrocœle grow out and extend outgrowths to the right and left, so as to roof over what would be the ambulacral grooves and thus form the epineural canals. The perihæmal canals also arise from hollow inter-radial outgrowths of the left posterior cœlom, except that the first arises, as in *Asterina gibbosa,* from the axial sinus. The arms of the larva gradually disappear, except the two postero-lateral, which are only cast off when the larva has sunk to the bottom and metamorphosis is complete.

LIST OF PAPERS CITED.

1. MacBride, E. W. "Some Points in the Development of *Ophiothrix fragilis,*" 'Proc. American Soc. of Zoologists,' Philadelphia, 1903.
2. Grave, Caswell. "*Ophiura brevispina,*" 'Mem. from Biol. Lab. Johns Hopkins,' 45.
3. Masterman, A. "The Early Development of *Cribrella oculata*, with Remarks on Echinoderm Development," 'Trans. Roy. Soc. Edin.,' vol. 40, Part 2.
4. Goto, S. "The Metamorphosis of *Asterias pallida*, with Special Reference to the Fate of the Body Cavities," 'Contributions from the Zool. Lab. of Mus. of Comp. Zool. at Harvard,' No. 88.

Croonian Lecture.—*On the Structural Constituents of the Nucleus, and their Relation to the Organisation of the Individual.*

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The latter half of the nineteenth century witnessed three events of supreme importance to biology. The first of these consisted in that reasoned theory of the mode of origin of new species with which the name of Charles Darwin will always remain associated. The second lay in the discovery, made by Strasburger in 1875, that the nucleus is not only a permanent organ of the cell, but that certain definite constituents of it are transmitted in unbroken sequence from one cell generation to another. Thirdly, Oscar Hertwig, also in 1875, showed that fertilisation consists not only in the union of male and female cells, but that the union of the two nuclei forms an essential part of the process.

At the present time, when evolutionary problems are being attacked at their very roots by the experimental study of variation, results are being accumulated which are capable of being dealt with from a cytological standpoint. Much is to be expected from a joining together of the forces engaged on what are really only different aspects of the same problem. What we really want to know is the nature and mode of working of the machinery which is responsible for the appearance of the characters manifested, as well as inherited, by the organism. We also are concerned with the nature of those inner changes which find their outward expression in what we designate as variation.

Whilst I do not of course pretend that all these questions can be satisfactorily answered at present, so many new facts which bear upon them have come to light within recent times that I venture to indicate some of what I conceive to be the more important results that have been obtained.

Every animal and plant either consists of a single cell, or it is made up of a cell-colony the members of which have had a common origin, inasmuch as they have all sprung from one mother-cell, the egg. And it is in the cells that the properties of an individual must ultimately be sought.

The more closely the cell is studied, the more irresistibly are we compelled to admit the supreme importance of the nucleus in directing and controlling its metabolic activities. Experiments have abundantly proved that if a nucleated protoplasmic mass be divided by artificial means, so that one portion contains, while the other lacks, the nucleus, the former alone is able to continue to discharge the ordinary vital functions, and to manifest those chemical and physical properties which necessarily underlie them. The enucleated fragment, on the other hand, although it may persist for some time, nevertheless loses, more or less rapidly, all those special faculties which should enable it to maintain a separate existence. Synthetic and secretory activities disappear, and death sooner or later supervenes.

Seeing, then, that the nucleus plays so fundamental a part in maintaining the due sequence of chemical change in the cell, the question naturally arises as to whether all those properties of the individual which find their expression in form, and in other externally recognisable characters, may not be attributed to the interaction of the nucleus with the cytoplasm external to it; whether they are not merely the inevitable result and outcome of the series of material changes that succeed each other within the confines of the constituent cells. I believe the answer must be in the affirmative. At the present time, however, such a general statement has but little value beyond expressing the general direction in which the many lines of investigations appear to be advancing. We are not likely, within the near future, to be in a position to trace all the stages which intervene between the importing of raw material and the turning out of the finished fabric. Modern research clearly indicates that the proximate problems are more intricate than they were formerly supposed to be. Nevertheless, I venture to think that the very complexity of the regulatory processes emphasises, rather than diminishes, the probability that a comprehensive physical explanation of organisation and inheritance will one day fall within our reach. But the final synthesis can only be made possible when we have understood the details, when the more immediate problems have been clearly stated and successfully solved.

Some of these problems are already in process of solution; thus numerous investigators during the last 30 years have done much to clear up the essential features of fertilisation, although it cannot be said that as yet there exists complete agreement on all points. For while it has been generally conceded since Hertwig's discovery, already alluded to, that the nuclear, and not the cytoplasmic, association constitutes the significant part of the process, there are still some who hold that the fusion of the cytoplasm is of direct and material importance in the transmission of the hereditary properties of the individual.

As this is a matter of great weight, an examination of the facts yielded by a study of fertilisation is demanded. It is true that the male gamete carries with it into the egg a small amount of cytoplasm. On penetrating the egg, the nucleus escapes from its cytoplasmic investment which then disintegrates and mixes with that of the egg, whilst the two nuclei approximate and often fuse. Although the amount of cytoplasm thus introduced is exceedingly minute, as compared with that of the egg, probably no one at the present time would regard this circumstance as *necessarily* implying a correspondingly small degree of importance, unless other evidence pointed also to the same conclusion.

Boveri has shown that when an enucleated fragment of the egg of one species of echinoderm is fertilised by the sperm of another species, or even genus, the resulting larva resembles the male parent. These statements have been sometimes challenged, but the explanation of the discrepant results obtained by different observers probably lies in the fact that the eggs themselves may be readily stimulated to develop parthenogenetically. Enucleated fragments of eggs already in this condition, even in the earliest stages, will have already received a developmental stimulus of a material nature from their own (*i.e.*, the female) nucleus. This must inevitably produce an effect on the final result. The recent discovery* that if eggs which are *known* to be in the early stages of parthenogenetic development are fertilised by the sperm of another species, the offspring actually do show a markedly increased resemblance to the maternal parent, is of the highest theoretical interest in connection with the mode of interaction between the nucleus and the cytoplasm.

The entrance of more than one sperm, and the fusion of their nuclei with that of the egg, is commonly followed by great disturbance in the course of development of the embryo, if, indeed, it is able to proceed at all. It is remarkable how very seldom polyspermy occurs in healthy cultures. I have myself examined many thousands of the eggs of Fucus, and I have only seen two or three examples of the entrance of more than one sperm into each egg. In some cases, however, polyspermy can be readily induced by anæsthetising, or in other ways interfering with the normal conditions of the egg; and the abnormal results that ensue serve to emphasise the correlation existing between nuclear constitution, and cell- and tissue-development.

The rarity of polyspermy, at any rate amongst the majority of plants, points to the existence of some means of actively preventing its occurrence in ordinary eggs. It is often stated that a cell wall is instantly excreted over the surface of the egg on fertilisation, and that in this way the entrance of additional sperms is prohibited. Such an explanation, if, indeed, it ever suffices, will not cover every case. In some eggs, as, for example, those of the Fucaceæ, the membrane is not formed till an appreciable time has elapsed after fertilisation. Hence the reason for the exclusion of the extra sperms must be sought in another direction. It seems probable that the cause of exclusion is connected with a sudden chemical change immediately resulting from the entrance of the first sperm into the cytoplasm of the egg. I shall show that in one case, at any rate, there is direct evidence of such a change in the reaction of the egg, and it may be remarked that it is quite intelligible that such a reaction should be affected or even arrested by anæsthetics, or other substances that interfere with the proper metabolism of the cell.

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* Curt Herbst, "Vererbungsstudien, IV," 'Arch. f. Entw.-Mech. d. Organismen,' vol. 22.

In the first place, it is certain that sperms are attracted to the egg by specific substances which are excreted from it and diffuse into the surrounding medium. Different sperms are affected by different substances, and what will attract those of one plant may be entirely without influence on those of another. For example, if two vaccine tubes, filled with very weak solutions of sugar and malic acid respectively, be placed in a vessel containing a mixture of the actively motile sperms of a moss and of a fern, those of the former will crowd into the tube containing the cane sugar, those of the fern into that filled with the solution of malic acid.

Since, then, the swarming of the sperms towards the egg is effected as a response to a definite chemiotactic stimulus, it is inherently probable that a correspondingly negative or repellant stimulus provides the egg with the means securing it against the entrance of more than one sperm. We have been able to show that this is the case in several of the Fucaceæ. If the large eggs of Halidrys be allowed to extrude into sea-water together with the sperms, the latter will, very shortly, be found to congregate on the surface of the egg. Each sperm possesses two cilia, with one of which it holds on to the egg, whilst with the other it lashes the water, endeavouring to force an entrance through the limiting layer of the protoplasm. So active are the movements that the relatively huge egg is seen to be rocked about in the water. In a short time a sudden change takes place in the egg. It swells, and then in a moment it becomes rugose or prickly first at one spot, and thence rapidly over its whole surface. At the same instant, all the sperms that were moving over its periphery rapidly leave it, and those that are unable to detach themselves sufficiently quickly are seen to become immediately paralysed and to die. Soon afterwards the egg shrinks in size and becomes quite smooth again. Later on a membrane is excreted over its surface, proving definitely that fertilisation has occurred.

In this instance, then, the case for the emission of a repellant, and even lethal, substance from the surface of the egg as the result of fertilisation is quite clear, and it perhaps diffuses from the fine mucilage-like threads that are shot out from the rugose periphery. Since making these observations on

Halidrys, I have been able, though not so easily, to detect the repellant effect in some other Fucaceæ. Still more recently, in an investigation on ferns involving the details of fertilisation, we have observed that after one sperm had entered the egg, the others close to its surface showed signs of greater disorganisation than those which were further removed from it. In this instance also, then, it seems clear that the entrance of a sperm produces a chemical change in the egg which is reflected over its whole surface, although we are not as yet in a position to make any definite statement as to the precise nature of the substance concerned.

It is another question whether the change is produced directly in the cytoplasm, or whether it emanates in the first place from the nucleus. Although certain facts seem at first sight to favour the latter view, I think the former is the more correct one. In many gymnosperms the two sperms which are formed in the pollen tube are both extruded into the egg. But only one of them is functional; the other soon disintegrates in the cytoplasm, and does not approach the egg-nucleus. But it must be borne in mind that the sperms are rather passively *shot into* the egg from the pollen-tube, than individually *attracted* into it. In Cycads, however, where the sperms are actively motile, only one enters the egg as a rule, and when more do succeed in penetrating it, they always perish at once. It may be suggested that as the egg of these plants is of enormous size as compared with that of the Fucaceæ, the change which is produced instantaneously in the latter may take longer to complete in the case of the gymnosperms. In any event it *does* take place, and the destruction of the additional sperms occurs in time to prevent them getting near the nucleus of the egg. Further evidence in favour of the cytoplasmic origin of the repellant substance is again afforded by Halidrys. It often happens that the eggs, as they are being shed, are broken into two pieces, of which one is destitute of a nucleus. Such fragments, however, as Williams has shown, commonly attract the sperms, and on the entrance of one of them the same repellant influence on the rest is as evident as in the case of normal eggs. Wilson, also, found conclusive evidence to show that when enucleated fragments of echinoderm-eggs are fertilised, only a single sperm is concerned in the process.

These observations seem to me to prove that the essential act of fertilisation consists not only in the union of two, and not more than two, nuclei, but also in the retention by them of a definite *structural basis*. For we have seen that the mere addition of nuclear *material* cannot be regarded as the important act, inasmuch as any extra sperms entering the egg are without influence on development, prejudicial or otherwise, provided that, as in the gymnosperms, they are destroyed so that their structural individuality is lost. That the egg-cytoplasm is essential as building-material is, of course, not disputed, and in so far as it consists of different substances, these will have different prospective values in the final result. In the case of many animal eggs, the cytoplasm shows physical differences in its various regions, and there is reason to think that this diversity in material is, at any rate sometimes, connected with differences in the structures finally to be developed. If, then, the egg be mutilated, so that a definite kind of raw material is abstracted from it, an imperfect embryo might be looked for, unless the removed raw material could be regenerated. The nucleus, as architect, would otherwise only be able to build a body correspondingly defective in certain parts. But such considerations as these do not weaken the position of the nucleus as the agent responsible for the particular direction and control of development which is followed by a cell, or, collectively, by a group of cells. In short, just as we have the strongest grounds for associating secretory and other metabolic activities of the cell with the nucleus as the prime mover, so, also, various lines of evidence indicate the same body, or rather certain constituents of it, as guiding the course of those chemical transformations in the cell and in the organism which make themselves apparent in characters such as form, colour, and the like, which can be appreciated by our senses.

I have indicated the possibility that certain constituents of the nucleus, rather than this body as a whole, may be charged with the control and direction of cellular development. It remains therefore to consider the evidence on which such a view, which in some form or another has often been put forward, is based. Darwin, Weismann, De Vries, and others, have each suggested the existence of particles which are responsible for the characters of the individual. And I may at once say that, apart from some such material units, I am unable to understand how the large mass of facts of which we are now in possession can be explained.

Within recent times our knowledge respecting the results of breeding, and also respecting the details of cellular organisation, has been greatly widened, and I believe that the evidence thus drawn from two entirely different sources irresistibly points to the same conclusion, and that the existence of discrete units which the breeder, on purely theoretical grounds, has been compelled to assume, is entirely borne out by the observed presence of certain structures which exist in vast numbers in the nucleus. These structures enter into the composition of those remarkable bodies, the chromosomes, which form such striking objects in the nucleus at the periods of division. Of course, I need not say that the particles in question are not to.

be regarded as the immediate characters themselves; they may, perhaps, be roughly compared with that class of bodies known as ferments, setting up in the extra-nuclear cytoplasm those changes which, in their sum-total, constitute development, and producing those chemical re-arrangements of which form, colour, etc., are the visible expression.

It will be convenient, in the first place, to examine the results obtained from experiments in cross-breeding, a field in which Bateson and others have achieved so great a measure of success. We can afterwards try to relate these results with the salient facts of nuclear structure which have been elucidated within recent times.

If two pure parents which differ in, say, one character from each other be chosen and mated, the offspring almost always behaves in a definite and uniform manner with regard to the character in question. Often it appears as if they all inherited the character of one parent only, that of the other having been entirely lost. But on allowing the hybrids to interbreed, it soon becomes plain that the alternative character was only latent, and not lost at all. It reappears in a fixed percentage of the offspring, usually to the extent of 25 per cent. When these individuals, thus exhibiting the recessive character, are mated, they continue to breed true, and the alternative dominant character, which replaces the recessive one in 75 per cent. of the individuals of that generation, never reappears amongst the recessive progeny so long as they interbreed only with one another.

As regards the individuals which make up 75 per cent. of the second (the f2) generation, it is found that whilst all of them show the dominant character exclusively, only one-third continues to breed true, whilst two-thirds of them, when mated together, prove to be hybrids; and in the next generation these continue to throw out one-quarter pure dominants, one-quarter pure recessives, and one-half hybrids. This is a remarkable result, and although it is not always directly or obviously obtained, it occurs in so large a number of instances as to prove sufficiently that the characters in question do behave as independent units. It is not surprising that there should be apparent exceptions to the rule; some of them have already been shown to be due to an incomplete analysis of the real characters themselves, so that composite features have been mistaken for units. But, at any rate, in the presence of so large a number of cases which *do* conform to the Mendelian rule, they may be safely left on one side so far as our present purpose is concerned, which is to find an explanation of the numerous positive instances that are everywhere recognised.

It is possible to make certain statements, which almost partake of the nature of inevitable conclusions, from a consideration of the mode of behaviour of those alternative characters so well termed *allelomorphs* by Bateson. In the first place, it is difficult to escape from the inference that the unit characters must be due to *material primordia* which are ultimately responsible for their appearance, and that these several primordia have a separate and persistent individuality of their own. The union of the primordia at fertilisation, as judged by the results, rather resembles a mechanical mixture than the formation of a chemical compound.

Inasmuch as two sexual nuclei unite at fertilisation, each one carrying with it characters of that parent from which it originated, it is clear that each sexual cell or gamete must possess, in its nucleus, half the whole lot of the primordia that will be contained in a fertilised egg. Furthermore, the manner in which the characters are distributed amongst the members of any one generation is such that no sexual cell can contain more than a single individual of any given pair of allelomorphs which are present in the body of the organism producing the sexual cells. This circumstance is expressed by the term "Purity of gametes," and it implies that, as regards any particular character, the primordium contributed by one parent only will be present in a gamete, the other primordium or allelomorph having been distributed to another gamete. When two gametes unite having identical primordia of any character, the resulting offspring must be pure bred as regards that character, and their progeny, if interbred, will keep true. But this is only so for each *separate* character; the *different* characters behave quite independently of one another. Thus, if we take A*a* as representing one pair of allelomorphs, and B*b* as representing another distinct pair, no gamete can contain A together with *a*, or B together with *b*, but it can contain *either* member of each pair, *i.e.*, AB, A*b*, *a*B, or *ab*. When two of these gametes unite, it can easily be seen that the characters will be combined as I have already stated.

The assumption as to the purity of gametes, which is in accordance with the facts it attempts to explain, is in harmony with cytological observations, and therefore may be accepted as representing more than a mere working hypothesis.

The clear results of statistical investigations of this kind seem to me to completely dispose of a view of fertilisation, otherwise quite possible, as being of the nature of a chemical union of the fusing nuclei. The facts conclusively point to the inference that there is only a mechanical mixture of the structural units contained in each of the sexual nuclei that take part in the act of fertilisation; the units retain their own identity, and they are again sorted out, though in different combinations, in connection with the differentiation of the sexual cells for the next generation.

We may now turn to consider the structure of the nucleus, and enquire into the nature of the evidence for the existence of permanent structures within it which will agree with what seems to be demanded by the facts of heredity as elucidated by experiments on breeding.

The resting nucleus is externally limited by a membrane, within which is contained a gelatinous, more or less reticulated, framework, the linin; and this is bathed in a more fluid medium, the paralinin. There are also one or more spherical bodies, the nucleoli, which form storehouses of materials that become separated and used up during times of nuclear activity. As long as the nucleus is in the resting condition, it betrays little of that intricate structure which it exhibits at periods of its division. Much of the complexity apparent at those times must be attributed to temporary physical changes proceeding in the colloidal masses which are then undergoing rapid change of state; but underlying these there is a permanent structural configuration which is constantly preserved and is a determining factor in controlling the course of those cellular changes on which the organisation of the individual ultimately depends.

When a nucleus is about to enter on division, the linin becomes impregnated with a substance termed chromatin, belonging to the nuclein class of compounds. The chromatin, or its precursors, is mainly to be found in the nucleoli as long as the nucleus is at rest; it is only during the period of division that it becomes aggregated in the linin. But the chromatin is not *evenly* distributed through the linin. It appears as very regularly arranged discontinuous swellings in the thread. These small swellings, formed of chromatin and linin, have been called *chromomeres.* The linin, made up of alternate discs of chromatin- and non-chromatin-containing segments, now either forms a convoluted filament, which finally breaks up into a definite number of segments, the *chromosomes;* or else these bodies are visible at the first onset of division as discontinuous areas of linin mapped out by the accumulation of chromatin in groups of chromomeres. The chromosomes are commonly stated to be constant in number for a given organism, but this is certainly not universally true, especially in the vegetative, or somatic cells. On the other hand, in the reproductive cells the number is on the whole very uniform, and even peculiarities of form distinguishing certain chromosomes may continually recur.

The chromosomes then take up definite positions at the equator of the nucleus, and each chromomere in the thread has already split into two exactly similar halves. This carries with it the longitudinal fission of each chromosome as a whole, and the two halves then move apart and are finally distributed, one to each of the two daughter nuclei which are thus formed.

The chromosomes are the only parts of the original nucleus of which it can be certainly affirmed that they are equally shared between the two daughter-nuclei. Seeing, then, that the net result of the process is to accurately divide the chromosomes (and consequently the chromomeres) by longitudinal fission, it would seem to be immaterial whether the actual numbers of these bodies remained constant or not, at any rate in the somatic divisions. The uniformity so commonly observed, especially when coupled with the occasional departure from it, probably indicates that the number typically present is to be taken as an expression of the organising function of the cell as a whole, rather than as evidence of the independent nature of the chromosomes themselves.* And this point of view is intelligible when we reflect that they are themselves only organised bundles of chromomeres.

At fertilisation, two nuclei unite, and their chromosomes consequently mingle. The immediate descendants of the cell thus produced are all similarly provided with double the number of chromosomes contained in the nuclei of the sexual cells before fusion occurred. It is, of course, clear that, in any organism, a limit to such duplication must soon be reached. It is found, as a matter of fact, that each act of fertilisation, with its concomitant doubling of the chromosomes, is associated with a correlative process of reduction to one-half. To this process the name of *Meiosis*† has been given. The way in which meiosis is effected is surprisingly uniform in both animals and plants, and its physiological importance may be gathered from the fact that, whilst it occurs in every organism that reproduces itself sexually, it is not bound up with morphological periods in the life history. It antedates all morphological differentiation, and is only *directly* related to sexuality, of the continuance of which it is both a consequence and a condition.

The great theoretical interest attaching to the phenomenon of meiosis lies in the convincing proof which it affords of the existence of persistent *structural* units which are directly responsible for the appearance of the various characters manifested by the developing organism.

It was formerly held by many investigators that the significance of fertilisation consisted merely in doubling the amount of chromosome-substance, and that reduction merely paved the way for this addition of bulk. The mass was supposed to act as a whole, rather than to be made up of separate and independent units. All the results of recent investigations have tended to upset this view, and to prove that just as the body itself is made up of individual cells, each an entity in itself, so also, and even more emphatically, the permanent elements of the nucleus are structurally distinct and physiologically diverse.

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* Much has been made of the fact that the chromosomes of successive cell generations can sometimes be almost certainly identified with those that went into rest during the previous telophase. But it should be remembered that such may easily happen, especially when divisions succeed each other rapidly, for the chromosome substratum chiefly consists of colloidal substance, which would only slowly lose its individuality under quiescent conditions.

† Farmer and Moore, 'Quart. Journ. Micr. Sci.,' vol. 48.

Reduction or meiosis is no mere halving of the bulk of the chromosome-substance; its significance is to be discerned rather in the sorting out and distribution between two daughter cells of the structural entities—the primordia of characters—which are handed on from one generation to another.

We may now follow, as briefly as possible, the way in which the chromosomes behave during the meiotic divisions, and contrast the process with that which obtains during an ordinary or somatic fission.

In the first place it is observed that as the nucleus advances towards division, and the chromosomes are able to be identified within it, their number is only half of that characteristic of the nuclei of the preceding cell generations that have arisen from the fertilised egg. A careful study of the facts has shown that this reduction is as yet only a false one, and that the real process is far more complicated. For each apparent chromosome is in reality made up of *two* chromosomes which have become temporarily united together. This union is, for a time, very intimate, and we have good grounds for saying that it is not mere chance which determines which two particular chromosomes shall unite to form a pair. For example, it may happen that two chromosomes in a premeiotic nucleus are different from the rest. Sometimes there are several dissimilar pairs. At meiosis, the pairing always takes place in such a way that like joins with like, to form the pseudo-chromosomes, as these bodies have been well called.

During the earlier stages, each of the paired chromosomes divides longitudinally, as in an ordinary somatic division, but the two halves do not yet become separated from one another. When the time comes for the splitting asunder of the two daughter-chromosomes to form the two nuclei, we find that the paired individuals now disunite and travel apart. When this has taken place, and the two daughter nuclei have been formed, the longitudinal fission, to which I have already referred, finally takes effect, and the two nuclei at once proceed to divide again. Each chromosome splits along the line of cleavage already marked out during the previous division. The process essentially resembles that of a somatic fission, but with this important difference, *viz.*, that the *reduced* number of chromosomes is retained. And this feature continues to mark all the nuclei of the post-meiotic cells, until the number is again doubled at the next act of fertilisation.

The net result of the meiotic phase is to effect the reduction in the number of chromosomes, and hence of the chromomeres, by the device of distributing half of the entire duplicated stock to one, and half to the other, nucleus. I would remark, in passing, that this fact points to the conclusion that the real importance of the chromosomes lies more in their *structure* as bundles of chromomeres than in their material composition alone.

I have referred to the peculiar circumstance that *similar* chromosomes pair to form the pseudo-chromosomes. It is, of course, evident that one of each of them will pass into the daughter-cells at meiosis, and hence ultimately into the nuclei of the gametes, which are the direct descendants of these cells. But it is also evident that when the gametes unite at fertilisation, not only will the chromosomes be doubled in number, but that the peculiar ones when present at all will again occur in pairs. The special interest of this lies in the indication that the chromosomes which pair at meiosis are derived from the male and female parents respectively.* This being so, meiosis is seen to be invested with profound significance in relation to the sorting out of the structural elements originally contributed by the parents, and the distribution of entire sets of them, but *in different combinations*, into the sexual cells. Thus the facts of meiosis are seen to fall completely into line with the conclusions drawn from experiments on breeding as far as the numerical distribution of characters is concerned.

That this pairing of the chromosomes is a real fact, and not a mere fanciful interpretation put on a complex series of phenomena, is abundantly proved by the work of the last few years. I will give two examples in illustration. It is well known that hybrids are frequently sterile, and this is often found to be associated with the failure on the part of the organism to accomplish the reduction of the chromosomes satisfactorily at the meiotic phase. This may happen if the chromosomes contributed by the two parents are unequal in number, and Rosenberg showed that it occurs in the hybrid between two of our common Sundews. *Drosera rotundifolia* has 10, and *D. longifolia* has 20 chromosomes in the sexual nuclei. When these two species are crossed, the somatic cells of the hybrid will, of course, exhibit 30 (10 + 20) chromosomes in their nuclei. Hence, at meiosis, it might be expected that there would be 15 pseudo-chromosomes produced. Instead of this, the number is seen to be 20. But, on examination, the 20 prove to be made up of 10 large and 10 small ones. What has happened is that the 20 contributed by the one parent, as well as the 10 furnished by the other, have retained their autonomy, but the 10 from *D. rotundifolia* have united with 10 of the 20 belonging to *D. longifolia*. The small ones represent the remaining 10, for which there were no mates. The statement that at meiosis the pseudo-chromosomes are composed of individuals derived from the male and female parents respectively could hardly receive stronger support.

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* I have purposely avoided discussing in detail the peculiar heterochromosomes that have been observed by Wilson and others in insects, as we are not at present in possession of sufficient evidence to fully estimate their possible significance.

The second example I will take from a hybrid fern which is at present under investigation in my laboratory. The plant in question, *Polypodium Schneideri,* is the result of a cross between *P. aureum* and *P. vulgare* var. *elegantissimum.* The nuclei of the former contain 35 chromosomes after meiosis, whilst *P. elegantissimum* has about 97 or 98. The chromosomes of the hybrid, at meiosis, instead of exhibiting a mean between the two parents, *i.e.* 66, has a much larger number. There is a little variation in the actual numbers, but the average lies between 90 and 100. That is to say, the quantity characteristic of the *elegantissimum* parent is retained. Besides this, we find at meiosis a large number of small, unpaired, chromosomes; these obviously represent the surplus over and above those furnished by the *aureum* parent. At division they betray their real nature by passing in their entirety to one of the daughter-nuclei, when these are formed.

Facts such as those I have recounted seem to emphasise the permanent individuality of the chromosomes, and indeed they have often been appealed to in this sense. The case is further strengthened by the not unfrequent occurrence in cells, before they have undergone meiosis, of one or more pairs of chromosomes that are different from the others, a circumstance to which I have already alluded.

Moreover, the mode of sorting out, and the general behaviour of the chromosomes throughout the cell-generations of the organism, are at first sight in striking accord with the results of statistical investigations on heredity, and they are looked upon by many people as representing the primordia of the characters which we have already considered.

But I believe there is a fatal objection to this point of view, and one which conclusively proves that we cannot regard the *chromosome itself* as the structural unit responsible for the characters of the organism. The objection is based on their relatively small numbers, and it was urged, though in a somewhat different connection, by Weismann, many years ago. I will illustrate my meaning by one example, and I wish to express my thanks to my friend, Mr. A. D. Darbishire, for kindly collecting the facts of the case. In the two pure races of *Pisum sativum* and *P. arvense,* there are no less than 18 pairs of characters respectively which in the hybrids behave as allelomorphs. Since, however, there are only seven chromosomes in the nuclei of the gametes, it is evident that the 18 allelomorphs could not all occur independently of each other, but that, if the chromosome be adopted as the unit, the allelomorphs would appear in at most seven groups, and further, that the members of each group would always appear in association.

Now such a correlation does sometimes occur in respect of a few characters, but it becomes relatively insignificant when the total number of independent pairs of allelomorphs is borne in mind. And if this causes difficulty in dealing with relatively few characters, it obviously gathers in force when the vast numbers of features which distinguish an organism are remembered.

But if for the chromosomes we substitute the *chromomeres* as representing the discrete units which are responsible for the production of the hereditary characters, the last objection loses its force. The chromomeres, consisting of special chromatin-containing linin bodies, are present in great quantities; so numerous that, as far as I am aware, they have never been counted in a single chromosome. They fulfil the condition of being distinct entities; and also, at meiosis, the divergent chromosomes contain, or rather are made up of, chromosomes arranged in two opposing groups, and derived from different parents.

At the same time, I am aware that there are certain difficulties introduced by this suggested substitution of the chromomeres for the chromosomes, though I think these are more apparent than real.

The cardinal facts which we have to take into account are: first, the large number of the allelomorphs and, secondly, their mutual independence. These two circumstances, taken together, are irreconcilable with a view that would regard the allelomorphs as invariably assigned to particular chromosomes. But if we attach to the chromomeres, or to any other still smaller particles, the properties of separate character-producing substances, the widely entertained view as to the real structural persistence of the chromosomes themselves will require some modification. For in order to give that complete independence observed to exist between most of the allelomorphs, it is clear that any given chromosome must be correspondingly indifferent as to which chromomeres enter into its composition.* This, however, amounts to a surrender of the doctrine that there exists any permanent structural arrangement in an individual chromosome lasting from one generation to another.

They might, perhaps, be compared with the hands that are successively dealt out from a pack of cards: each new hand, in respect of the number of cards, may resemble, but is not really identical with, those of the preceding deals. So, too, the chromosomes which reappear at each division would be similar to, but not *necessarily* the same as, those of the preceding division. The material particles of which they are built up are shuffled in the intervals elapsing between one division and another. This might well happen without any change of form; just as in some crystals, for example, certain atoms or groups of atoms may be replaced without altering the crystalline form. In the organic world, we know that the precise form of a particular organ may be produced, although the cells of which it is made up may not be those which should have entered into its composition. I need only refer to the experiments proving that a substitution of blastomeres may take place in the early stages of development of the frog's egg without affecting the subsequent differentiation of the tissues. Again, I might refer to the replacement of the crystalline lens, when extirpated from a salamander's eye, by a new lens formed not from ectodermal, but from mesodermal, cells. And, as a matter of fact, the abundant anastomoses which the linin exhibits during the earliest stages of nuclear division seem to provide just that mechanical condition for distribution which theory requires. This anastomosis has always been a difficulty in the face of assumptions of chromosomal permanence.

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* In the heterotype pseudochromosomes, each member must, however, be composed of homologous primordia contributed by the male and female parent respectively. It may well be that this is the significance of synapsis, which forms so characteristic a feature of the heterotype mitosis.

The chromosomes, then, would represent similarly *organised* groups of chromomeres, but they would *not* necessarily represent permanent or persistent structures in the sense that each one is to be looked on as being invariably composed of the same chromomeres. Their constancy in form and number would be the expression of *organisation* within the cell, and not of an unchanging aggregation of the *same constituents.*

The relation between the chromosomes and the origin of them from the male or female parent respectively of course remains unaffected; what evidence we possess points to the conclusion that the two sets of chromosomes, and consequently their chromomeres, remain distinct in all the cell-generations up to meiosis.* In a number of animals and plants they reappear in two distinct groups at every nuclear division during the earlier stages of ontogeny; and Blackman has shown, for the Uredineæ, that the nuclear approximation which corresponds to fertilisation is not really consummated until many cell-generations afterwards; not, indeed, until the meiotic phase comes on. In these plants, then, the chromosomal isolation is carried to an extreme, for the sexual nuclei do not unite during the somatic phase to form a single nucleus; they co-exist side by side as separate bodies, although they periodically divide in a synchronous fashion.

In suggesting the chromomeres as the agents which are responsible for the production of the characters, I am aware that I may be laying myself open to the charge of merely retreating from a position never very strong, and now beginning to prove untenable, to another that will perhaps turn out to be no less vulnerable. But I think we are bound to take into account the structures we can actually see, and the evolutions of which we can definitely follow, before we take refuge in other and merely hypothetical entities. At any rate, the plain facts of heredity, on the one hand, seem to postulate the existence of large numbers of real units in the cell in order to account for the observed phenomena, and, on the other hand, a study of the cell shows that such structures are really present, although they may be crowded together into larger or smaller distinct packets. And this will continue to be true, whether the chromomeres, or still smaller particles, are adopted as the units.

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* Even if it should be found that this distinctness is definitely lost in the premeiotic nuclei, it would not weaken the strong evidence in favour of the shuffling of the primordia, and their re-arrangement in groups of homologous pairs, at meiosis.

This view as to the variable nature of the chromosomes seems to me to render intelligible certain other facts which have often proved puzzling, and have become especially so during some recent investigations on the cytology of ferns. In studying the nuclei of a series of varieties that have sprung, and are still originating, from some of our common species, *e.g.*, the Male Fern, one cannot fail to be struck by the somewhat unexpected occurrence of chromosome-numbers which show a great, but quite irregular, deviation from that characteristic of the type.

Sometimes the new numbers are in excess, sometimes they fall below that which one would have anticipated. Thus in two varieties of the Male Fern known as *Polydactyla*, the numbers in the prothallial cells are 66 and 90 respectively. The two ferns are remarkably alike in external features, as is indicated by their common name; but they are known to have originated as distinct plants. In spite of their similarity, they show many points of important difference when the various stages of their life-histories are compared. They are both apogamous, and both replace the normal act of fertilisation by the union of nuclei derived from adjacent vegetative cells, in spite of the fact that they produce perfectly active sperms. One of them, however, entirely lacks the female organ—the archegonium—whilst in the other, though present, it is quite functionless. Along with these peculiar features there is associated the change in the number of the chromosomes already referred to. Now, it is important to notice that the differences do not form multiples either of each other or of the number (72) of the typical fern from which they are known to have originated. And when other varieties are brought into the comparison, all grades of deviation are shown. It would thus appear, since there is no corresponding diversity in the sizes of the individual chromosomes, as though the variation must have arisen through a *re-arrangement of the whole substance* of which they are made up; in other words, the chromomeres have become thrown into new combinations quite apart from any further modifications they may have undergone. The consideration of such an example as this serves to illustrate the truth of the general statement that the chromosomes, like other organs, are subject to change, both as to size and number. Such a difference is not uncommon between closely allied species; and in several of the ferns to which I have referred, the number is not even constant in the same individual. It would seem, then, that the bodies in question form rather a frail support for the heavy weight of speculation that has been piled upon them.

Much of the importance which has been attached to the chromosomes in connection with organisation is connected with the fact that in the plants the process of meiosis is not directly followed, as it is in animals, by the differentiation of sexual cells. In most plants the meiotic phase forms the starting point of a definite stage in the life-history of the organism, and all the cells are distinguished by the possession of the half, or the post-meiotic, number of chromosomes. Thus in the fern, the plant as we know it, possesses the double set. Meiosis is associated with the formation of the spores, and when these germinate they give rise to the sexual plant, the prothallium. All the cell-nuclei of this structure continue to show the *reduced* or *post-meiotic* number of chromosomes as the consequence of meiosis. It seemed natural, then, to regard this change of nuclear structure as connected in some causal way with that alternation of a sexual and a sexless generation which is so striking a feature in the vegetable kingdom, especially in the higher classes, of which the ferns may be taken as examples.

The investigations on apogamy, and especially on apospory, have shown this view to be untenable. Indeed, it has been known for some years that eggs of species of Echinoderms when stimulated to develop parthenogenetically proceed to segment on perfectly normal lines in so far as they can develop at all. Their nuclei retain the post-meiotic number of chromosomes throughout the short life of the early larval stages, that is up to the death of the embryonic animals.

We have been able to show, in the case of ferns, that in apospory there may be a direct transition from the fern plant to the prothallium, accompanied by the *total suppression* of the meiotic phase which normally marks the passage from the one generation to the other. The nuclear character of such an aposporous prothallium exactly resembles that of the fern plant from which it sprang, instead of only having half the chromosomes. The chief difference of importance which the prothallium itself exhibits, as contrasted with an ordinary one, lies in its inability to produce fertilised eggs. The sexual organs may be fully formed, and the sperms are frequently found to be vigorously motile. Fertilisation, however, never occurs, and the fern either arises directly from the unfertilised egg, or from the tissues of the prothallium. In such a case, then, the entire life-history is passed through without any reduction of the chromosomes; the egg, like the other cells of the prothallium, already contains the full complement, which normally is not supplied until fertilisation. There is, therefore, no reason to be surprised at the omission of fertilisation in these cases.

It is sometimes possible to induce prothallia which are normal as regards their nuclear contents, *i.e.*, are reduced, to produce embryos apogamously. But in all these instances, excepting one, we have found that the normal sexual fusion, which is here prevented from taking place, is replaced by a fusion of nuclei from adjacent vegetative cells. In this way the normal doubling of the chromosomes is secured. This circumstance has been used as an argument to prove that at any rate the double set is necessary for the production of the sporophyte generation.

We have encountered one instance, however, in which there seems no room for doubt that the fern plant sprang from a prothallium of *post-meiotic* structure, without any preparatory nuclear fusion. The plant in question* appeared in a close fernery, and, from what we know of its history, it must have started as a spore. Presumably, therefore, it had passed through meiosis, and this is borne out by the small number of chromosomes present in its nuclei. From the prothallium, fern plants were produced apogamously, and they are all characterised by the possession of the *reduced, i.e.*, the post-meiotic, number of chromosomes.

It thus becomes evident that, given a *complete set of normal* chromosomes, whether in single or in duplicate, the entire life-history *may* be passed through. The peculiar fusions between the nuclei of adjacent prothallial cells, together with the extreme rarity of the formation of the fern plant without the double set, may perhaps be taken to indicate some *normal* relation as existing between the duplicate number and the sporophyte, but it clearly cannot be of any fundamental importance.

The fact that both stages of the life-history can be gone through in the absence of that nuclear change which ordinarily corresponds to each one of them is a proof that the organisation of the species, whatever may be the phases through which it passes, can be completely effected *either* with the single, *or* with the double, set of chromosomes; but the meaning attached to sexuality, as importing into the organism the means of producing variation, becomes even more clearly defined than before.

I desire, in conclusion, to obviate a possible misapprehension which, perhaps, might arise from the way in which I have spoken of the chromomeres as the agents responsible for the appearance of the characters manifested during ontogeny. Of course, in calling them *primordia,* I only mean to imply that they are the agents that determine the particular sequence of chemical changes which shall occur in the unstable cytoplasm. I do *not* mean that they are themselves the characters *in parvo.* The very fact that, as agents, they may persist in a dormant state, and thus fail to excite the appearance of the characters appropriate to them, as may happen in the concurrent presence of dominant and recessive allelomorphs, sufficiently emphasises this. For as long as the course of metabolism can be completely impelled by one of the two allelomorphs, the influence of the other will remain latent. We may, perhaps, find an analogy in the behaviour of *Penicillium,* which, although it is provided with a powerful battery of ferments, does not indiscriminately attack all the nutrient materials of the substratum when plentifully provided with suitable carbohydrates.

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* *Lastrea pseudo-mas cristata apospora*, see 'Annals of Botany,' vol. 21, p. 180.

Such an example serves also to illustrate another aspect of the problem, and one that perhaps often meets with less attention than it deserves. For admitting that there are good grounds for assigning the production of hereditary characters to the action of discrete bodies, we are still without an explanation of the obvious facts of functional correlation and adaptation. The environment, in the widest sense of the term, often determines *which* of the many potential characters of a cell shall actually develop.

We know something as to the manner in which this may be achieved in certain instances. For example, nutritional, as well as other and more obscure, stimuli were found by Klebs to determine the particular one out of several alternative courses of development which was followed in the case of certain algæ. And again, the Hormones of Starling may be quoted as types of substances, apparently of relatively simple chemical structure, which serve as excitors of specific cellular activities in the complex animal body.

The conceptions of *primordia* which are responsible for the appearance of hereditary characters on the one hand, and also of *specific exciting substances* on the other, seem to be both necessary, and the one to supplement the other.

Every organism and every cell has larger potentialities than are ever realised in any single ontogeny. The hereditary mechanism imposes the limits within which development *can* take place; but within those limits other conditions may determine the path actually followed.

On Certain Phenomena of Inactivation and of Inhibition exhibited by Precipitin Antisera.

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(From the Physiological and Pathological Laboratories of the University of Sydney.)

*General Phenomena of Inactivation and of Inhibition exhibited by Heated Antisera.**

When a precipitin antiserum, prepared with blood serum or with egg white, is heated to 75° C. for 10 or 30 minutes, it loses its power of yielding a precipitate with the homologous protein (inactivation), and it acquires the power, when added in sufficient proportion, of preventing the formation of the precipitate that would otherwise appear when the homologous protein interacts with unheated antiserum (inhibition). An identical heat exposure does not produce the same degree of inhibitory power in different homologous antisera, the inhibitory capacity being estimated by the amount of unheated antiserum required to elicit a precipitate.

In the majority of the antisera tested, inhibition was complete, that is, no precipitation occurred, when (*a*) 0·1 c.c. heated antiserum, (*b*) 0·1 c.c. unheated antiserum, and (*c*) 0·001 c.c. or 0·005 c.c. homologous protein were allowed simultaneously to interact, or when (*a*) was mixed with either (*b*) or (*c*) for 24 hours before the other ingredient was added. Inhibition was always complete when the amount of the heated antiserum was three times that of the unheated antiserum, and always incomplete when these proportions were reversed. Incomplete inhibition was manifested by delay in appearance or by diminution in amount of the precipitate, or by both phenomena.

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* Our conclusions are based on more than 1000 separate interactions, involving 20 different antisera, about 50 different combinations of heated and unheated antiser having been tested. The antisera were prepared in the rabbit by intraperitoneal injections of blood serum or of egg white. The general procedure was the same as that indicated in a previous communication ('Roy. Soc. Proc.,' B, vol. 78, p. 298, 1906), and most of the interacting substances were employed in a fresh undried condition. To obviate coagulation, the antisera were diluted with two or four volumes of water before being heated, but this water is not included in the amounts of heated antiserum to which reference is made in the text. Thus 0·1 c.c. heated antiserum indicates 0·1 c.c. antiserum heated together with two or four volumes of water. We ascertained that the additional water did not noticeably affect the interaction.

When complete inhibition was established, it did not cease with lapse of time, nor was it overcome by any excess of homologous protein, but it could be overcome (that is a precipitate would appear) on a suitable addition of unheated antiserum, or on a smaller addition of unheated antiserum together with homologous protein.

When, as occasionally happened, 0·1 c.c. heated antiserum was inadequate to prevent precipitation in a mixture of 0·001 c.c. or 0·005 c.c. homologous protein and 0·1 c.c. unheated antiserum simultaneously added, it was invariably also inadequate when allowed to stand in contact with the unheated antiserum for 24 hours before homologous protein was added, but it was frequently adequate to prevent precipitation when allowed to remain in contact with the homologous protein 24 hours before the unheated antiserum was added. In view of the fact that no excess of homologous protein could overcome any inhibition, it is futile to conclude from such evidence that the heated antiserum antagonises either the homologous protein or the unheated antiserum more than the other. As a matter of fact, its influence is probably exerted not so much on either of the interacting substances as on the product of their interaction (*v. infra*).

Analogous results have been obtained by other observers, notably by Müller,* in respect of lactosera, and by Eisenberg.†

Relation between Inactivation and Inhibition.

On the hypothesis that precipitins constitute, on Ehrlich's scheme, receptors of the second order, equipped with a relatively unstable ergophorous and a relatively stable haptophorous affinity, inactivation is produced by destruction of the former, while inhibition is due to retention of the latter alone. Any factor that would abolish the ergophorous affinity would, *ipso facto*, render the precipitin inactive and inhibitory, so long as the haptophorous affinity was not thereby damaged. Precipitin would thus become transformed into precipitoid. In other words, inactivation and inhibition would be merely different aspects of the same phenomenon, and this is the accepted relationship between them (Eisenberg, Müller, and others).

We have evidence, however, that inactivation and inhibition are separable phenomena, that inactivation does not, in the above circumstances, necessarily imply inhibition, so that the relation between them does not admit of so simple a statement. Thus a precipitin antiserum (No. 38) prepared with hen-egg white, when heated to 70° C. for 30 minutes, did not become noticeably inactive or inhibitory. When heated to 72° C. for 10 minutes, it was rendered almost completely inactive, but not noticeably inhibitory. Exposure to 72° C. for 30 minutes produced complete inactivation without, however, any appreciable inhibition. Further exposure to 75° C. of a portion of the antiserum already heated to 72° C. for 30 minutes yielded a solution still wholly inactive, but now strongly inhibitory. We have repeated these observations with concordant results.

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* Müller, 'Archiv f. Hygien.,' vol. 44, p. 126, 1902.

† Eisenberg, 'Centralb. f. Bakt.' (Originale), vol. 31, p. 773, 1902.

It is noteworthy that the conversion of antiserum from the active to the inactive condition appears to be a temperature change rather than a time effect, and that inactivation occurs rapidly at the temperature at which we succeeded in obtaining, in an acidulated solution of a homologous antiserum, a heat coagulum not found in natural rabbit serum.*

A second means of effecting a separation of the phenomena of inactivation and of inhibition was accidentally revealed. Another homologous antiserum (No. 11), prepared in the rabbit by injections of hen-egg white, had been stored in a dry condition. At the expiry of 11 months it was found to be still readily soluble in salt solution, but to have become wholly inactive, no trace of precipitate appearing on interaction with homologous protein. When 0·1 c.c. of this unheated inactive antiserum solution† was allowed to interact with an equal volume (0·1 c.c.) of active homologous antiserum (Nos. 23 and 25 were separately employed) and with homologous protein, no appreciable inhibition could be detected. When, however, a triple volume (0·3 c.c.) of the inactive antiserum solution interacted with a single volume (0·1 c.c.) of the active antiserum in otherwise similar circumstances, the full precipitate was in every instance obtained, but in three out of six tubes its appearance was delayed for about 24 hours. When it is borne in mind that three volumes (0·3 c.c.) of any antiserum heated to 75° C. was invariably found to completely inhibit precipitation when one volume (0·1 c.c.) of a different but homologous antiserum interacted with homologous protein, it must be admitted that any inhibition effected by the unheated inactive antiserum (No. 11) was uncertain and exceedingly slight. It is, indeed, possible that the occasional delay in the appearance of the full precipitate may have been due to some slight non-specific inhibition exerted by increased serum concentration.

When, however, the inactive antiserum (No. 11) was heated to 75° C. for 30 minutes, it developed a pronounced inhibitory capacity—so much so that

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* Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 309, 1906.

† 0·1 c.c. of the inactive antiserum solution contained 0·01 gramme dried antiserum (No. 11) and was, therefore, roughly equivalent to 0·1 c.c. undried antiserum.

0·1 c.c. heated antiserum mixed with homologous protein for 48 hours was able to prevent precipitation when 0·1 c.c. active antiserum (No. 23 or No. 25) was subsequently added. The inhibition was constant, but incomplete, when 0·1 c.c. heated antiserum was mixed with 0·1 c.c. active antiserum (No. 25) and homologous protein was simultaneously or subsequently added. The inhibition was complete when 0·2 c.c. heated antiserum interacted with 0·1 c.c. active antiserum and homologous protein, and was independent of the order in which the substances were added.

Both by heating to 72° C. and by spontaneous degeneration, therefore, it was possible to obtain antisera which were wholly inactive and yet not at all or only doubtfully inhibitory, and thus to effect a separation of the phenomena of inactivation and of inhibition. In both instances, also, heating to 75° C. so altered the constitution of the inactive, non-inhibitory antisera, that they came to acquire powerful inhibitory properties.

If inactivation and inhibition were inseparable phenomena, it might be supposed that inactivation was merely a particular manifestation of inhibition, that precipitin was not necessarily altered by heating to 75° C., but that an independent inhibitory substance was developed at that temperature. Our observations render this supposition untenable.

The mere separation of the phenomena of inactivation and of inhibition might be reconciled with the commonly accepted statement of Ehrlich's theory of the constitution of precipitins by the twofold assumption of a diminution, followed by an increase, in the combining affinities of the haptophores. It might, for example, be assumed that an immediate effect of the weakening or destruction of the ergophorous affinities, resulting in inactivation, would be some weakening of the haptophorous affinities also, whereby molecules of the nature of epi-precipitoid, or precipiton, would be produced. On the analogy of epi-toxoids, or toxons, such epi-precipitoids, or precipitons, having feebler combining affinities than the unaltered precipitin molecules, would be readily evicted by them, and so might give rise to little or no noticeable inhibition of the precipitate. It would be necessary also to assume that heating to 75° C. restored or enhanced the potency of the haptophores, so that epi-precipitoids were transformed into syn-precipitoids or even into pro-precipitoids, having combining affinities respectively equal to or greater than those of unaltered precipitins. We do not see how the presence of epi-precipitoid in the inactive, non-inhibitory antisera described by us can be either proved or disproved, unless the occasional doubtful inhibition above noted is to be taken as an indication of its presence.

Other facts, however, render this explanation inadequate. It has been shown that, after the interaction of 0·001 c.c. or 0·005 c.c. homologous protein and 0·1 c.c. antiserum, the addition of further quantities of antiserum produces a fresh deposit proportional to each added mass of precipitin.* This is strongly suggestive that, after each interaction, unoccupied binding affinities of the homologous protein are available. Further, if inhibition is produced by the binding of the thermostable haptophorous groups of the heated antiserum to receptors of the protein molecule, it is remarkable that the addition of considerable masses of homologous protein fails to provide receptors for the precipitin groups of the unheated antiserum and to bring to an end the inhibition.

All that we can say is that inactivation and inhibition are separable phenomena, commonly appearing together because of the proximity of their respective temperatures of origin, and depending on substances whose interactions do not admit of so simple an expression as that afforded by the usual statement of Ehrlich's theory.

Specificity of the Inhibition exercised by Heated Antisera: Phenomena of "Crossed" Inhibition.

The non-appearance of a precipitate in an otherwise effective precipitin interaction is not always due to the same conjunction of chemical and physical factors, and of these factors some exert an inhibitory influence that is altogether non-specific. But the inhibitory power of heated antiserum is markedly specific, probably not less specific than the reaction of the corresponding unheated antiserum. The nature of this specificity is best illustrated by a concrete example. Thus, a heated hen-egg antiserum will prevent the formation of a precipitum between hen-egg albumin and any hen-egg antiserum, but not between ostrich-egg albumin and ostrich-egg antiserum, nor will it have any effect on non-avian precipitin interactions. The phenomena of "crossed" inhibition are revealed by the fact that heated hen-egg antiserum will inhibit precipitation between hen-egg albumin and ostrich-egg antiserum and between ostrich-egg albumin and any hen-egg antiserum. Moreover, when ostrich-egg proteins were substituted for the corresponding hen-egg proteins in each of the above experiments, identical results were obtained. These results may be summarised in the following Table I:—

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* Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 305, 1906.

Table I.

No.	Homologous protein, 0·005 c.c.	Active antiserum, 0·1 c.c.	Heated antiserum, 0·1 c.c.	Precipitation.
1	Hen-egg white	Hen-egg	Hen-egg	Inhibited
2	Ostrich-egg white	Ostrich-egg	,,	Unaffected
3	Horse serum	Horse	,,	,,
4	Hen-egg white	Ostrich-egg	,,	Inhibited
5	Ostrich-egg white	Hen-egg	,,	,,
6	,, ,,	Ostrich-egg	Ostrich-egg	,,
7	Hen-egg white	Hen-egg	,,	Unaffected
8	Horse serum	Horse	,,	,,
9	Ostrich-egg white	Hen-egg white	,,	Inhibited
10	Hen-egg white	Ostrich-egg	,,	,,

The Nature of inhibition as indicated by the Specific Solubility of Precipitates in Heated Homologous Antisera.

Müller* was the first and, so far as we are aware, the only observer to point out that a precipitate resulting from a precipitin interaction was soluble in the heated antiserum, but his observation had reference only to lactosera. The possible significance of this phenomenon led us to undertake a more extended series of tests, in order to determine how far this property was possessed by other heated antisera, and how far the capacity for dissolving precipitate might be identified with the capacity for inhibition.

Our experiments gave the following results:—(1) The precipitum formed between hen-egg albumin and hen-egg antiserum is soluble in any hen-egg antiserum heated to 75° C., but not in ostrich-egg antiserum, nor in horse antiserum, similarly heated; (2) the precipitum formed between ostrich-egg albumin and ostrich-egg antiserum is soluble in ostrich-egg antiserum heated to 75° C., but not in hen-egg antiserum, nor in horse antiserum, similarly heated; (3) the precipitum formed between horse serum and horse antiserum is soluble in any horse antiserum heated to 75° C., but not in hen-egg or ostrich-egg antiserum similarly heated; (4) such precipita are insoluble in water, in salt solution, in natural rabbit serum, and in natural rabbit serum heated to 75° C., all of which are likewise non-inhibitory; (5) the amounts of heated antisera used in dissolving the precipitates were the same as those used in producing inhibition (from 0·1 to 0·3 c.c.), and where the lesser quantity was unable to inhibit, it was also found to be unable to dissolve the precipitate. When a precipitate was soluble in heated antiserum, it rarely happened that the whole of the deposit was taken up into solution, but by far the greater portion was so taken up, only a small trace being left in marked contrast to the original deposit, and in marked contrast also to the full deposit which reappeared when a precipitate was shaken up with any heated antiserum other than that strictly homologous. It seems legitimate to draw the conclusion that the capacity for dissolving precipitate is a specific property of inhibitory antisera.

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* Müller, *loc. cit.*, p. 175.

This conclusion is reinforced by the results of testing the solubilities of the precipitates obtained in "crossed" interactions, as indicated in Table II, and by comparison of these results with the "crossed" inhibitions shown in Table I.

Table II.

No.	Homologous protein, 0·005 c.c.	Active antiserum, 0·1 c.c.	Precipitate after 24 hours.	Heated antiserum, 0·1 c.c., added to precipitate.	Result to precipitate.
			mm.		
1	Hen-egg white	Hen-egg	2·0	Hen-egg	Dissolved
2	Ostrich-egg white	Ostrich-egg	3·0	"	Unaffected
3	Horse serum	Horse	1·5	"	"
4	Hen-egg white	Ostrich-egg	0·5	"	Dissolved
5	Ostrich-egg white	Hen-egg	0·5	"	"
6	" "	Ostrich-egg	3·0	Ostrich-egg	"
7	Hen-egg white	Hen-egg	2·0	"	Unaffected
8	Horse serum	Horse	1·5	"	"
9	Ostrich-egg white	Hen-egg	0·5	"	Dissolved
10	Hen-egg white	Ostrich-egg	0·5	"	"

These results suggest that the precipitum given by hen-egg antiserum and ostrich or any egg albumin, other than hen-egg albumin, may be regarded as similar to that produced by ostrich-egg antiserum and any egg albumin other than ostrich-egg albumin. We may assume this precipitum to result from the general avian character, or component, of the proteins used in the immunisation, while the greatly increased precipitum produced by hen-egg antiserum and hen-egg albumin, or by ostrich-egg antiserum and ostrich-egg albumin, we may assume to be due to the specific hen or ostrich character, or component, of the protein used for injection. A heated-egg antiserum would thus inhibit and dissolve the general avian precipitum plus its own strictly homologous precipitum, but would neither inhibit nor dissolve the non-homologous component of any precipitum, however closely related. This principle is probably of general application, and might be of value in the practical differentiation of closely related proteins.

The phenomena above described emphasise the close relationship that exists between the capacity for solution and the capacity for inhibition of precipitate, but we have still more striking evidence to offer. For example, an antiserum rendered inactive, but non-inhibitory, by heating to 72° C., has no power of dissolving precipitate, but when the antiserum is rendered inhibitory, by heating to 75° C., it coincidently acquires the power of solution. Antiserum (No. 11), rendered inactive, but non-inhibitory, by long-continued desiccation, did not dissolve precipitate, but when heated to 75° C., and thereby made capable of inhibiting, it also became capable of dissolving precipitate. When antisera are mixed with a sufficiency of homologous protein, their precipitable content may be entirely thrown out of solution and the antisera rendered inactive. Such inactivated antisera, when heated, do not gain the power of preventing precipitation (a corresponding result was obtained by Müller in respect of lactosera), and neither do they gain the power of dissolving precipita.

Further, an antiserum rendered inhibitory and solvent, by heating to 75° C., has almost completely lost both capacities after heating to 85° C. for 10 minutes, and has completely lost both by exposure to 85° C. for 30 minutes, or to 100° C. for five minutes. Such destruction of inhibitory and solvent capacity does not reactivate the antiserum, nor does the addition of heated or unheated natural rabbit serum restore its inhibitory or solvent capacity.

Notwithstanding many experiments of the above nature, we found it impossible to dissociate the capacity for inhibition from the capacity for solution of precipitate. Both appear and disappear together, and it would seem not unreasonable to draw the further conclusion that an antiserum acquires the power of preventing precipitation in virtue of its acquiring the power of dissolving precipita; in other words, that an essential part of the mechanism of specific inhibition is the development of a no less specific capacity for solution of precipitate.

Conclusions.

1. Inactivation and inhibition are separable phenomena, the former appearing without the latter in hen-egg antisera heated to 72° C., or exposed to long-continued desiccation, the latter not becoming appreciable until the antisera are heated to 75° C.

2. Inhibition and solution of precipitate by heated antisera are specific inseparable phenomena.

3. The specific capacity for dissolving precipitate acquired by heated antisera is probably an essential part of the mechanism of specific inhibition.

4. Certain phenomena of precipitin reactions, including precipitation, inactivation, inhibition, and solution of precipitates, appear to be incompatible with the commonly accepted statement of Ehrlich's theory of the substances concerned in precipitin interactions.

Miadesmia membranacea, *Bertrand; a New Palæozoic Lycopod with a Seed-like Structure.*

By M. Benson, D.Sc., F.L.S.

(Communicated by Dr. D. H. Scott, F.R.S. Received April 26,—Read June 13, 1907.)

(Abstract.)

The vegetative organs of this interesting new type were discovered by Bertrand in 1894. He found them in sections of a calcite nodule from the Gannister beds of Hough Hill, England. A large quantity of new material has become available, and now not only are more details known as to the vegetative organs, but a fairly complete knowledge of the reproductive organs is possible.

Miadesmia was exceedingly minute, its stem slender and without any trace of skeletal tissue. It is the first Palæozoic Lycopod of herbaceous character known structurally. The megasporophylls which were identified by Dr. D. H. Scott, F.R.S., in 1901, show a more advanced type of seed habit than has hitherto been met with in Cryptogams. The megasporange gives rise to but one thin-walled spore, which in development and structure resembles an embryo sac and germinates *in situ*. An integument surrounds the sporange, leaving but a small orifice as micropyle. This is surrounded by numerous long processes of the integument, which formed a collecting and incubating apparatus for the microspores. There is no trace of an envelope about the microsporange. The carpellary leaf was shed at maturity, and resembles a winged seed.

Disregarding the structural modifications of the megasporophyll, the nearest affinity of Miadesmia among forms so far known seems to be with the non-specialised species of Selaginella, such as *Selaginella selaginoides*, but the foliage leaves show the archaic leaf-base comparable with that of Lepidodendreæ.

On the Identification of Chitin by its Physical Constants.

By Igerna B. J. Sollas, Fellow of Newnham College, Cambridge.

(Communicated by Professor W. J. Sollas, F.R.S. Received May 27,—
Read June 13, 1907.)

The determination of the distribution of chitin in the animal kingdom is hampered by the absence of any test or positive means of identifying it. Gamgee, in his 'Text-book of Physiological Chemistry,' gives a list of structures of invertebrate animals in which chitin has been described. But when those cases are eliminated in which the identification has been based solely on the negative character of insolubility in caustic alkalis or weak acid, the revised list, as it appears, for instance, in von Fürth's 'Vergleichende chemische Physiologie der niederen Tiere,' is greatly curtailed. It is true that chitin yields a characteristic decomposition product, the amido-derivative of sugar known as chitosamin, in definite proportions, but the amount of material available is not always sufficient to allow of the preparation of this product. In cases, however, where we have other reasons for suspecting the presence of chitin, the reducing action of the chitosamin resulting from the treatment of the original substance with sulphuric acid is a valuable confirmatory test.

Under these circumstances it seemed that it might be worth while to make a determination of the specific gravity of chitin by the well-known method of a diffusion column. The specific gravity of any substance as thus determined stands in real and intimate relation to its chemical constitution, for the mass dealt with is always small and can therefore be rendered homogeneous by various cleansing processes. It is readily permeated by the suspending fluid and the absence of any other sources of error may be ensured without difficulty.

In addition to the determination of the specific gravity, that of the refractive index should be made, for two substances may possess the same specific gravity, but not the same chemical composition, though when we have other reasons for suspecting their identity this is unlikely.

The refractive index of any substance has been shown by Gladstone and others to be connected with the specific gravity by a relation which, whether expressed as $\frac{n-1}{d}$ or more correctly as $\frac{n^2-1}{(n^2+2)d}$, is constant for that substance and is independent of the temperature. Therefore the demonstration of common values for the specific gravity and the refractive index of two substances affords strong evidence of their identity.*

Unfortunately, I do not possess a reading microscope, so that I have taken my readings direct from a scale at the back of the experimental tube. But I have appreciated the advantage of the use of glass beads: when these are made and suitable fluids chosen the process need occupy but little time.

The fluids I have used are chloroform, of which the specific gravity is roughly 1·48 at 21° C., and "absolute" alcohol of specific gravity 0·791 at 21° C. The specific gravity of chitin from the integuments of Astacus having been found roughly to lie somewhere between 1·39 and 1·41, mixtures of chloroform and alcohol of specific gravity 1·36 and 1·44 were made with the help of a Westphal's balance, and these mixtures formed the two fluids of the column. In using light fluids it is not possible to float glass beads, such as are used for dealing with minerals in heavy fluid columns, and which are described in 'Nature':† it is necessary in the present case to blow the beads dumbbell-shaped, but with both ends hollow. Hence it is possible to obtain beads which float with their long axis horizontal and this is an advantage, for the nearer the axis of the bead approaches to the horizontal position the less is likely to be the error which arises in estimating its centre of gravity.

Chitin from various sources was prepared in the usual way by soaking in 10-per-cent. hydrochloric acid, boiling in 5-per-cent. caustic potash‡ for many hours with frequent changes of the liquid, washing in water, very dilute hydrochloric acid, and extracting in alcohol and ether. In some cases this treatment did not render the chitin quite colourless: the last traces of pigment were removed by Mayer's method of bleaching, in which chlorine is the active agent.

The specific gravity of chitin from integuments of Astacus cleaned in this way was found to range between 1·400 and 1·404. The chitin of a caterpillar, *Bombyx cynthia*, was slightly heavier, 1·404 to 1·408, of the cockroach wing 1·402, of the pupa case of the buff-tip moth 1·404: of Oniscus and spiders, again, the value agreed very nearly with that of Astacus. Two species of Myriapoda gave the value 1·397 to 1·399; one of these was the common English Lithobius, the other a large millepede which I picked up in the Karroo, South Africa, but have not identified.

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* This method was originally published in 1885 by Professor Sollas in the 'Roy. Soc. Dublin Proc.,' new ser., vol. 4, p. 378, and subsequently in 'Nature,' 1891, vol. 43, p. 404, and 'Quart. Journ. Geol. Soc.,' vol. 58, p. 163, 1902.

† *Loc. cit.*

‡ The use of potash of this strength, which is weaker than that employed by Krukenberg, was recommended to me by Dr. F. Gowland Hopkins, to whom I am indebted for much kindly interest in this investigation.

The chitin which had the lowest specific gravity of any I have measured was that of Limulus and of Scorpio. This had a value 1·393 when cleaned as above described. If, however, stronger potash is used, chitin from Astacus and from Limulus approach each other in a diffusion column and give a zone at a level corresponding to a specific gravity 1·398. This figure seems to represent the value of the specific gravity of pure chitin, for, as stated below, it is that of chitin precipitated from its solution in strong acid.

The strength of potash which gives a common value for the specific gravity of chitin from both Limulus and Astacus is 40 per cent. It must be used for a short time only after the usual long boiling in 5 or 10 per cent., as the prolonged action of strong potash causes chitin to become soluble in weak acid.

Neither boiling in 50-per-cent. hydrochloric acid nor soaking in strong hydrofluoric acid alters the specific gravity of chitin.

Young chitin from a crayfish which had recently undergone ecdysis was heavier than the older skin; it formed a zone at a level corresponding to a specific gravity of 1·410, with outlying pieces down to 1·420. Unfortunately, I have not obtained the specific gravity of the cast skin. It proved to be more readily soluble in weak acid after boiling potash than the younger chitin and, not foreseeing this, I inadvertently dissolved it before I had taken measurements.

The refractive indices for red light of the chitin from various sources which I have measured lie between the limits 1·550 and 1·557. The measurement was made by Becke's method.* That from Astacus and Limulus integuments lies between 1·554 and 1·557. The fluids used in these determinations were aniseed oil and mixtures of aniseed and fennel oils calculated to have the refractive indices 1·550 and 1·557.

As stated above, chitin was precipitated from its solution in strong hydrochloric acid, in order to determine which of the obtained values, ranging between the extremes 1·393 and 1·408, represents the specific gravity of pure chitin.

The solution was performed in a refrigerator, carapaces of Astacus and Limulus being used. At the end of a fortnight no difference in the bulk of the solid was noticeable on inspection. The acid was then poured off and diluted in the refrigerator with 10 times its volume of water cooled till a thin film of ice formed on the surface. A copious milky precipitate was formed. Fresh acid was poured over the remaining pieces of chitin, and slight precipitates obtained at intervals of two days from this freshly added acid. The solution in hydrochloric acid, when kept at a temperature of less than 0° C., is thus seen to be not unstable. The precipitate, which was flocculent, was washed by decantation until an acid reaction was no longer obtained; it was then boiled in alcohol and ether. Placed in a specific gravity column, it formed a broad zone, extending from 1·380 to 1·390. Some of the precipitate was then dried at a low temperature (40° to 60° C.). It formed a thin film on the evaporating dish, which was scraped off with a scalpel, and then formed a white powder. This powder had a specific gravity 1·398, and this, I conclude, is the specific gravity of chitin. If dried too rapidly at a higher temperature than 60°, the precipitate flakes off the dish and its specific gravity is no longer uniform.

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* "Ueber die Bestimmbarkeit der Gesteinsgemengtheile auf Grund ihres Lichtbrechungsvermögens," 'Sitzungsber. der K. Akad. der Wissensch.,' Vienna, vol. 102, p. 358, 1893.

The substances of unknown chemical composition which I have examined by this method are Lumbricus bristles, the organic portion of the molluscan radula, the pupal skin of Pieris, and of some other Lepidoptera stated by Griffiths to be "pupin." I had hoped to deal also with the skeletal substance of the branchial bars of Amphioxus, which are stated to be insoluble in alkalis, but I could obtain no residue after boiling the pharynx, even in weak caustic potash. An account of the specific gravity of the organic portion of the shell of Sepia, in which chitin has been found by Krukenberg, is also given below. Lumbricus chætæ were isolated in the following manner:—The worm was slit up along the mid-dorsal line and the alimentary canal and its contents removed. The remaining body wall was then washed in a current of water to remove any matters which might have escaped in the process of removal of the gut. The contents of the gut are troublesome unless removed at once. The worms thus far cleaned are now treated with 10-per-cent. hydrochloric acid, and then boiled in potash as usual in the preparation of chitin. If after prolonged treatment with potash any matter other than chætæ remains undissolved, this is most readily got rid of at a later stage of the cleansing process (after extraction in alcohol) by separation in a diffusion column, say of chloroform and alcohol. The bristles will float on the chloroform, the insoluble dirt (presumably sand, undigested wood fibres, and cellulose, set free in removing the alimentary canal) sinks to the bottom. The bristles can then be removed with a pipette and extracted in ether. In this way a considerable quantity of chætæ can be collected with very little trouble. They form a neat zone in a diffusion column at a level corresponding to a specific gravity 1·392. They are doubly refracting. I have measured one index only, it lay between 1·557 and 1·550. I conclude, therefore, that the chætæ of Lumbricus contain chitin. This is in agreement with the results obtained by Goodrich.*

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* 'Quart. Journ. Micr. Sci.,' 1897, vol. 39, "Notes on Oligochætes."

From their solubilities and their behaviour with Millon's reagent and with the xanthoproteic test, Goodrich considered that the bristles of Oligochæta are evidently chitin or some nearly allied substance. But more recently Schepotieff,* after a lengthy research, concludes: "Diese Reaktionen zeigen, dass die Borsten jedenfalls nicht aus einer einheitlichen Substanz bestehen, sondern aus mindestens zwei. Die eine derselben zeigt die Reaktionen der Eiweisskörper. Die andere kann schwerlich Chitin sein, wie der Mangel der Zuckerbildung bei Behandlung mit Schwefelsäure ergiebt." With regard to the last difficulty, the absence of sugar formation after treatment with sulphuric acid, I am unable to find that it exists, for I obtained copious sugar reduction with Trommer's test. Schepotieff (p. 672) dissolved the bristles in 89-per-cent. sulphuric acid, kept them for 25 hours at a temperature of 40° to 50° C., diluted the solution with 10 times its bulk of water, and kept the diluted solution at 100° C. for some hours. He then neutralised with barium carbonate, and tested the solution with copper sulphate and sodium hydrate. It was suggested to me by my friend Miss Durham that the use of barium carbonate in place of the usual caustic potash for neutralisation may have led to Schepotieff's difficulty, as the precipitate, consequent on neutralisation with barium carbonate, would carry down the sugar with it. At any rate, sugar is certainly present after treatment with sulphuric acid, and there is no reason why one of the two constituents should not be chitin. That more than one substance should be present in the bristles is not surprising. I have not met with any chitinous skeleton which did not give the proteid reaction to which Schepotieff alludes before it was cleaned.

Pupa cases of *Pieris brassicæ* and *P. napi*, cleaned in the same way as chitin, had a specific gravity of 1·400 and refractive index between 1·554 and 1·557. Griffith† has examined these integuments chemically, and has stated that they consist of a new animal substance which he calls "pupin." He dissolved the skins (after they had been cleaned with potash, water, alcohol, and ether) in hydrochloric acid, and precipitated from this solution a substance to which he assigned the formula $C_{14}H_{20}N_2O_5$, and found that it split when boiled with strong mineral acids into leucin and carbon dioxide. Von Fürth remarks that the proportions of C, H, and O point to an albuminoid, but the N-content is strikingly small. He suggests that a closer investigation would be worth while, as he supposes that the products of splitting, besides leucin and carbon dioxide, must have been overlooked.

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* 'Zeitschr. Wiss. Zool.,' vol. 74, 1903, p. 674.

† 'Comptes Rendus,' II, vol. 105, pp. 320—321, and 'Bull. Acad. Roy. Belg.,' (3), vol. 24, 1892, p. 592.

I can only state that the substance has, like the integument of other Lepidopteran pupæ, the physical properties of chitin, and that I have obtained evidence of the presence of sugar in it after treatment with 90-per-cent. sulphuric acid at a temperature of 40° C. for 24 hours. The sugar was detected by Trommer's test, as in the other cases mentioned.* The molluscan radula I have studied from this point of view by this method chiefly in the case of the periwinkle, *Littorina littorea*, the limpet, *Patella vulgata*, and the whelk, *Buccinum undatum*. The radula of Patella, as I have shown elsewhere,† contains silica and iron in considerable quantities. To cleanse it we must, therefore, treat it for some time (24 hours) with strong hydrofluoric acid. A control experiment showed that the specific gravity of chitin from the integument of Astacus was unaltered by this treatment. After washing out the hydrofluoric acid the usual method of preparing chitin was used. The radulæ still retain a faint yellow colour after this treatment; they were placed in chlorine water, when they became colourless and somewhat transparent. They were cut into short lengths, and these were found to form a neat zone in the diffusion column at a level corresponding to a specific gravity 1·405 to 1·407. Littorina radulæ gave a closely similar result. In the case of Littorina I used the confirmatory test, and obtained reduction of copper salts by Trommer's method.

Buccinum radulæ differ from the others which were measured in that they form a broad irregular zone in a diffusion column. The extreme tips of the radulæ, taken from the radular sac, however, give a uniform specific gravity of 1·404. The remainder of the radulæ form a band extending from 1·400 to 1·411, or even lower. It is noticeable that the pieces in this band for the most part hang with their greater length vertical, and the oldest end of each length is lowermost. It, therefore, seems possible that some second resistent substance, in addition to chitin, is present in this case in increasing quantities as we pass forwards from the radular sac. If this is the correct interpretation of the facts, it is interesting in connection with the occurrence of free sulphuric acid in the salivary glands of Buccinum, and it is not surprising that an organ used to bore holes in shells with the aid of sulphuric acid should need to be specially resistent. For some time I fancied that the irregularities in the specific gravity of this radula might be due to small particles of sand caught between the bases of the teeth and the basal membrane. But the specific gravity of the teeth, when isolated

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* Since this was written, von Fürth and Russo have shown by chemical methods that the integuments of Lepidopteran pupæ consist of ordinary chitin, 'Beitr. z. chem. Physiol. u. Pathol.,' vol. 8, 1906.

† 'Quart. Journ. Micr. Sci.,' 1907, vol. 51, pp. 115—136.

from the basal membrane, shows that this cannot be so. The same radulæ of which I had measured the specific gravity were treated for a moment with strong hydrochloric acid, with the result that the basal membrane was instantly destroyed and the teeth freed; after washing and reboiling in alcohol they were found to have specific gravities of even wider range than that of the intact radula, the heaviest of them being 1·430. By soaking the radula of Buccinum for several weeks in very strong potash (above 40 per cent.) the teeth can be isolated, the basal membrane being destroyed. This fact, discovered by chance, aroused some doubt as to whether the basal membrane were really chitin, and a repetition of the experiment was made in which the skeletons of two segments of Astacus were subjected to the action of the same strength of potash. The two segments became isolated from one another, the abdominal feet (pleopoda) were also isolated from the segments, the soft connecting membranes being destroyed. And yet these membranes have the same specific gravity and the same refractive index as chitin, and no one has ever suggested that they are anything else. These membranes differ in staining reactions from the chitinous pieces which they connect in just the same way as the basal membrane of Buccinum differs from the teeth.

A third method of isolating these teeth is to soak the radula in hydrofluoric acid for 24 hours previous to the usual cleansing process. The teeth will then be liberated when the radula is boiled in 5-per-cent. potash solution. Teeth thus prepared gave the least range that I have found in this material; they formed in all cases a dense zone at the level 1·393; in one instance they were confined to this zone, but in two other columns a certain number of teeth were scattered down to 1·411, and a very few, perhaps five or six, hung at various depths below this. Astacus integuments subjected to precisely the same treatment had a specific gravity of 1·406; and, as happened when strong potash was used, the connecting membranes were destroyed. Some of the heavier Buccinum teeth were removed with a pipette from the column; in one or two of them a single large granule, apparently a foreign body, was visible: it appeared to have undergone partial solution. Since, in the case of Murex, I have seen obvious sand grains firmly embedded in the teeth of the radula, it would seem probable that the exceptionally heavy teeth are to be accounted for by accidents of this kind. The minor variations in specific gravity may perhaps be due to some second organic substance accumulated in the thick body of the tooth remote from the margin, and thus not affecting the measurement of the refractive index, which lies constantly between 1·550 and 1·554. The fact that the heavier teeth hang in the column with their fangs upwards is in accordance with this view.

The refractive indices of the organic portion of the Molluscan radula, which I have determined in Littorina, Patella, and Buccinum, lie between 1·550 and 1·554.

The shell of Sepia has been shown by chemists to contain chitin, and the percentage yield of chitosamin has been measured by them. After decalcification with 10-per-cent. hydrochloric acid and boiling in 5-per-cent. potash it readily breaks up, the thick outer wall of the shell with the rostrum and lateral expansions becoming freed from the mass of delicate septa. This outer layer quickly becomes transparent and colourless in parts, the rostrum and thickened region in its neighbourhood being yellowish. The septa remain opaque, they adhere to one another by a maze of low sinuous ridges, the capillary spaces between which readily become filled with air, unless special care is taken to avoid this. The specific gravity of the outer layer is not a constant quantity, but ranges from 1·385 to 1·393. Alternate boiling in various strengths of potash higher than 5 per cent. and in 50-per-cent. hydrochloric acid do not reduce this range. The septa, on the other hand, form a zone in the diffusion column at the level of chitin. The refractive index of the inner laminæ lies between 1·550 and 1·554; that of the outer is variable, lying sometimes between 1·550 and 1·544, sometimes between 1·550 and 1·554. The variable specific gravity of the outer layer of the Sepia shell points to the presence of more than one substance. As the change in specific gravity goes hand in hand with a corresponding change in refractive index, the suggestion naturally arises that there may be a series of chitins, of which a lighter and a heavier member are present in the Sepia shell.

Rosenheim* has given some reason to suppose that the fossil carapace of *Pterygotus osiliensis* contains chitin. It would be interesting to apply the method described in this paper in confirmation of his results, and to extend it to the study of other organic substances.

Summary.

The determination of the physical constants of chitin forms a useful method of identifying it. The specific gravity of chitin from various sources approximates to the value 1·398, a number which represents the specific gravity of chitin precipitated from its solution in strong acid. The refractive index lies between the limits 1·550 and 1·557.

The bristles of Lumbricus, the pupal skin of Pieris and other Lepidoptera, the radula of Mollusca and the shell of Sepia, when freed from mineral matter and easily soluble organic substances, have specific gravities and refractive indices which lie between the same limits as those of chitin from various sources.

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* 'Roy. Soc. Proc.,' B, vol. 76, 1905, pp. 398—400.

The Inhibitory Action upon Subsequent Phagocytosis, exerted on Active Normal Serum by Inactive Normal Serum through which Bacilli have been passed.

By J. C. G. Ledingham, M.A., M.B. (Aberdeen), Assistant Bacteriologist, Lister Institute.

(Communicated by Dr. C. J. Martin, F.R.S. Received April 30,—Read June 13, 1907.)

Considerable difference of opinion still prevails regarding the nature of the opsonic substances present in normal serum. Wright, Bulloch and Atkin, etc., uphold the view that the opsonin of normal serum is a simple thermolabile body. Muir, on the other hand, regards the opsonin as a body which behaves like complement, while Dean holds that it is essentially thermostable and in all probability co-operates in its action with a thermolabile complement. The demonstration of anti-bodies by complement-deviation experiments (Bordet, Gengou, Pfeiffer and Friedberger, etc.) has recently proved fruitful in connection with the bacteriolysins, hæmolysins, precipitins of immune sera, and the following experiments were designed to test whether, by a similar method applied to phagocytosis, the presence in normal serum of opsonic amboceptors could be demonstrated :—

Experiment I.

Normal human serum was heated for 30 mins. at 60° C. (denoted "A"). A very thick emulsion of tubercle bacilli in 1 : 1000 salt solution was added in equal volumes to "A" and kept in contact therewith for 1 hr. 30 mins. at 37° C. The mixture was then centrifugalised (7000 revolutions per minute) for 1 hr., and the supernatant fluid pipetted off (denoted "B").

Equal volumes of "B" and fresh normal human serum were now mixed and kept in contact at 37° C. for 1 hr. (final fluid denoted "T").

The steps employed in the preparation of the control fluid were essentially similar to the above. Thus, heated normal serum was added in equal volume to salt solution (instead of to tubercle emulsion) and the mixture kept in contact for 1 hr. 30 mins. at 37° C. Equal volumes of this mixture and fresh normal serum were now taken, and retained in contact for 1 hr. at 37° (control fluid so obtained denoted "C").

The opsonic contents of "T" and "C" were now compared in the usual way, fresh tubercle and staphylococcal emulsions being employed. The result was as follows :—

	Bacilli per leucocyte.
Test fluid "T" + leucocytes + *B. tuberculosis*	0·5
Control fluid "C" + leucocytes + *B. tuberculosis*.........	2·3

	Cocci per leucocyte.
Test fluid "T" + leucocytes + staphylococci	1·4
Control fluid "C" + leucocytes + staphylococci	3·57

The difference between "T" and "C" was sufficiently striking, but it seemed desirable to re-test the two fluids with stronger emulsions of *B. tuberculosis* and staphylococcus. Accordingly, after standing in the cold room over-night at a temperature of 2° C., they were again compared, with the following result:—

	Bacilli per leucocyte.
Test fluid "T" + leucocytes + *B. tuberculosis*	0·8
Control fluid "C" + leucocytes + *B. tuberculosis*	4·5

	Cocci per leucocyte.
Test fluid "T" + leucocytes + staphylococcus	3·9
Control fluid "C" + leucocytes + staphylococcus	24·5

Thus the ratio of "C" to "T" in the case of tubercle rose after 16 hrs. from 4·6 : 1 to 5·6 : 1, and in the case of staphylococcus from 2·5 : 1 to 6·2 : 1.

Experiment II.

Fluids "T" and "C" were prepared in the way described above and their opsonic contents compared. Result:—

	Bacilli per leucocyte.
Test fluid "T"	2·6
Control fluid "C"	6·0

	Cocci per leucocyte.
Test fluid "T"	4·2
Control fluid "C"	17·2

After standing over-night in the cold room, they were re-tested with fresh emulsions of bacilli and cocci. Result:—

	Bacilli per leucocyte.
Test fluid "T"	1·0
Control fluid "C"	7·7

	Cocci per leucocyte.
Test fluid "T"	2·3
Control fluid "C"	9·0

The ratio of "C" to "T" thus rose after 16 hrs. from 2·3 : 1 to 7·7 : 1 in the case of *B. tuberculosis*, while in the case of staphylococcus it remained practically the same.

Experiment III.

In this experiment the heated serum and the salt solution were emulsified directly with *B. tuberculosis* in an agate mortar. Result:—

	Bacilli per leucocyte.
Test fluid "T"	1·2
Control fluid "C"	5·0

	Cocci per leucocyte.
Test fluid "T"	5·4
Control fluid "C"	15·9

Experiment IV.

Instead of *B. tuberculosis*, staphylococci were brought in contact with heated normal serum. The further steps in the preparation of "T" and "C" remained the same. Result:—

	Cocci per leucocyte.
Test fluid "T"	10·7
Control fluid "C"	15·7

(Opsonic content with regard to *B. tuberculosis* omitted.)

The difference between "C" and "T" is thus relatively small. A similar experiment was made, in which staphylococci, which had been heated at 60° C. for 1 hr. 30 mins., were passed through normal heated serum. Result:—

	Cocci per leucocyte.
Test fluid "T"	20
Control fluid "C"	18

So far, then, as these latter experiments are concerned, it does not appear that much, if any, inhibitory action on fresh normal serum is exerted by heated serum through which staphylococci have been passed. In view of the consistent results obtained with tubercle bacilli in the first series of experiments, it was important to determine whether saline solutions, through which bacilli have been passed, exhibited a similar inhibitory action on fresh normal serum.

A saline solution (1 : 1000) was digested at 37° with tubercle bacilli for 1 hr. 30 mins. and then centrifugalised. The extract so obtained was added in equal volume to fresh normal serum and kept at 37° C. for 1 hr. A control fluid was made by digesting the normal serum with salt solution (1 : 1000). The contents in tubercular opsonin of these two fluids were then compared, with the following result:—

	Bacilli per leucocyte.
Test fluid	5·4
Control fluid	7·7

Three similar experiments yielded the following figures:—

	Bacilli per leucocyte.		Cocci per leucocyte.
Test fluid	6·7	5	11·9
Control fluid	10·6	6	11·9

Extracts of tubercle bacilli in salt solution have, therefore, little, if any, inhibitory action on the tubercular and staphylococcal opsonins of fresh normal serum.

Conclusions.

Heated normal serum, through which tubercle bacilli have been passed, exerts a marked inhibitory action on the tubercular and staphylococcal opsonins of fresh normal serum. Such an action points to the presence in normal serum of opsonic amboceptors, the combination of which with the free receptors thrown off from the tubercle bacilli deviates the complement from the fresh normal serum, and thus inhibits phagocytosis. Further experiments of this nature are in progress.

Contributions to our Knowledge of the Poison Plants of Western Australia.

By E. A. MANN, Government Analyst, and W. H. INCE, Ph.D., F.I.C.

(Communicated by Professor J. Norman Collie, F.R.S.)

(Abstract.)*

The State of Western Australia has for many years had an unenviable reputation by reason of the large numbers of indigenous plants which, by their poisonous properties, have caused heavy losses amongst cattle and other varieties of stock. At the present time these losses are estimated by the Agricultural Department at about £15,000 to £20,000 per annum.

Mr. Jas. Drummond, in 1842, was the first to make a comprehensive report on these plants. Since then other references may be found from time to time. In July, 1895, the West Australian Government Bureau of Agriculture proposed to initiate a full enquiry into the properties of these plants, and placed the matter in the hands of Mr. S. S. Dougall (Municipal Chemist).

Mr. Dougall's report is to be found in the 'Journal of the Department of Agriculture of Western Australia,' December, 1900. He reports that every effort to detect the presence of an alkaloid in the "York Road" and "Box" poison plants had been unsuccessful.

In 1899 the Western Australian Department of Agriculture sent specimens of these plants, together with the "Heart Leaf," to Professor Balfour, at Edinburgh, by whom they were forwarded to Professor Stockman, of the University of Glasgow, for chemical examination. Professor Stockman attributed the toxic principle to a substance belonging to the saponin series.†

In 1902, samples of several of these plants were again sent to England by the Department of Agriculture, and submitted to Professor Dunstan, F.R.S., of the Imperial Institute, for examination. A report was received (1905) on one only of the plants (the "Narrow Leaf"), in which Professor Dunstan stated that, by extraction with alcohol, he obtained "a yellow crystalline substance, probably identical with, or closely related to, quercitrin, the glucoside found in the bark of *Quercus tinctoria*."

As far as the present authors are concerned, the chemical investigation was

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* The full paper was communicated in two parts :—

Part I, dealing with Cygnine, was received February 14, 1906, and read February 22, 1906.

Part II, dealing with Lobine, was received November 5, 1906, and read December 13, 1906.

† 'Journal of the Department of Agriculture, Western Australia,' December, 1900.

begun by one of them in 1897, and continued intermittently for some years; it was not till 1905, however, that a systematic examination of these plants was commenced in the Government Laboratory.

Isolation of the Alkaloid from the "York Road" Poison Plant, Gastrolobium calycinum. *Cygnine,* $C_{19}H_{22}N_2O_3$.

One pound of the powdered plant was extracted with 76-per-cent. alcohol, rendered slightly acid with acetic acid. The bright green percolate was evaporated at 50° C., and the residue treated with 1-per-cent. sulphuric acid, filtered and the filtrate cleared with lead acetate, the excess of lead being removed by means of sulphuretted hydrogen. The cleared solution was neutralised and precipitated with tannic acid, the precipitate was collected and mixed with freshly prepared lead hydrate. This mixture was then carefully dried below 50° C. and extracted with hot 70-per-cent. alcohol. The alcoholic solution was evaporated and made acid with hydrochloric acid. From the solution a crop of colourless crystals separated. These were purified by recrystallisation. On injection into a guinea-pig, however, they exhibited no toxic action.

In the next experiment 10 lb. of the powdered plant were extracted with alcohol and treated as before, but with this difference, that in none of the operations was the temperature allowed to rise above 30° C.

The result was an indefinite crystalline mixture that under the microscope consisted of needle-shaped and cubical crystals. These were separated by careful solution in alcohol and evaporation *in vacuo.* (The cubical crystals are only slightly soluble in alcohol.) By this means a hydrochloride that crystallised in fine acicular crystals was obtained. That it possessed toxic properties was proved by the fact that one-tenth of a grain killed a guinea-pig in seven minutes. The cubical crystals, mentioned above, were often met with in later investigations; they are colourless, very soluble in water, but only slightly so in alcohol. They contain no nitrogen or chlorine and possess faintly acid properties. The mean of two analyses gave:—$C = 68{\cdot}7$ and $H = 8{\cdot}1$. Calculated for $C_{12}H_{16}O_3$, $C = 69{\cdot}2$, $H = 7{\cdot}7$. It seems probable that this substance is a decomposition product of the alkaloid, for whenever, during the extraction of the plant, a high temperature (100° C.) had been used for evaporation, the cubical crystals either preponderated or were the only crystalline substance obtained. Moreover, on warming the acicular crystals with water, for the purpose of recrystallisation, the result was always the same, an increasing crop of the cubical crystals and a non-crystallisable brown substance.

The acicular crystals were the hydrochloride of a nitrogenous substance, and they must be kept dry, otherwise they slowly decompose; moreover, in their preparation from the plant, it is absolutely necessary that in none of the operations should the temperature be raised above 30° C.

The free alkaloid is still more unstable, and, when set free from the hydrochloride by means of ammonia, is uncrystallisable and decomposes readily.

Hydrochloride of the Alkaloid.—The acicular crystals, when obtained pure, were analysed. The mean of seven analyses gave the following results:—$C = 62{\cdot}6$, $H = 6{\cdot}6$, $N = 7{\cdot}9$, $Cl = 10{\cdot}5$. Calculated for $C_{19}H_{22}N_2O_3HCl$, $C = 62{\cdot}9$, $H = 6{\cdot}3$, $N = 7{\cdot}7$, $Cl = 9{\cdot}8$.

The salt is not very soluble in water, 100 c.c. dissolving 1·932 grammes at 15° C. The aqueous solution gives the usual alkaloidal reactions.

From its chemical composition, behaviour, and physiological effect, this substance seems to be new to science and has been named "Cygnine."*

The gold salt was prepared by allowing a solution of the hydrochloride in alcohol, together with chloride of gold, to evaporate *in vacuo*. It crystallises in yellow needles, m. p. 220° C., with decomposition.

On analysis it yielded the following results:—mean of six analyses, $C = 34{\cdot}0$, $H = 3{\cdot}5$, $N = 4{\cdot}5$, $Cl = 21{\cdot}0$, $Au = 29{\cdot}7$. Calculated for $C_{19}H_{22}N_2O_3HAuCl_4$, $C = 34{\cdot}3$, $H = 3{\cdot}5$, $N = 4{\cdot}2$, $Cl = 21{\cdot}3$, $Au = 29{\cdot}5$.

The gold salt is fairly stable in a dry atmosphere, but gradually decomposes under the influence of light and moisture.

Cygnic Acid, $C_{10}H_{10}O_4$.—This acid was obtained from the lead precipitate obtained during the clearing of the alcoholic extracts. The precipitate was suspended in water, and treated with excess of sulphuretted hydrogen, and filtered. The solution was treated with barium hydrate, the resulting brown precipitate washed with alcohol, and decomposed with ammonium sulphate, filtered, and the filtrate evaporated on a water-bath. The residual crystalline mass was dissolved in a small quantity of water, reprecipitated as a crystalline powder by alcohol. This redissolved in water and, evaporated *in vacuo*, gave large colourless needles, which decomposed on heating. On analysis they gave:—mean of three analyses, $C = 52{\cdot}0$, $H = 7{\cdot}5$, $N = 12{\cdot}5$. Calculated for $C_{10}H_8O_4(NH_4)_2$, $C = 52{\cdot}6$, $H = 7{\cdot}0$, $N = 12{\cdot}3$.

From the ammonium salt the barium salt was prepared; on analysis it gave $Ba = 41{\cdot}0$, $C_{10}H_8O_4Ba = 41{\cdot}6$ of Ba. Attempts to prepare the pure acid from the barium salt in dilute sulphuric acid only gave a colourless amorphous residue. The acid is precipitated by lead, barium and silver salts. The silver salt easily reduces on heating, forming a mirror.

Gastrolobic Acid, $C_7H_{10}O_5.H_2O$.—It was noticed that a flocculent yellow precipitate was formed during that part of the process of clearing the alcoholic percolates from the powdered plant, which was partly effected by blowing air through the liquid to get rid of excess of sulphuretted hydrogen. This precipitate, after repeated recrystallisation from water and alcohol, and treating with decolourising agents, still remained yellow. It is soluble in alkalies with a deep yellow colour. The silver salt is a faint cream-white precipitate. The free substance contains no nitrogen of sulphur. It is a very weak acid, for the ammonium salt (red-yellow tufts of needles) decomposes in the air, losing ammonia and reverting to the original acid. The sodium salt is more stable (red-yellow tufts of needles). The acid decomposes without melting on heating. The mean of three analyses, C = 43·5, H = 6·3, H_2O = 10·7. Calculated for $C_7H_{10}O_5H_2O$, C = 43·7, H = 6·3, H_2O = 9·4.

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* Western Australia was originally known as the Swan River Settlement, and a black swan is still the emblem of the State.

The sodium salt gave Na = 19·6. Calculated for $C_7H_{10}O_6Na_2$, Na = 19·4.

Gastrolobin, a vegetable gelatinoid.—In clearing the syrupy percolate from the sulphuric acid extraction, it was noticed that, after the addition of lead acetate, the solution became quite limpid. To isolate the mucilaginous principles, the lead precipitate was washed, suspended in water, and decomposed with sulphuretted hydrogen. The filtrate had regained its viscous character. Absolute alcohol precipitated a flocculent substance, which was soluble in water. The substance does not reduce Fehling's solution and does not contain nitrogen or sulphur. The aqueous solution is precipitated by tannic acid, lead acetate, dilute sulphuric acid, and sodium carbonate. The substance was purified by solution in water and reprecipitation with alcohol (five times). An analysis was made: C = 44·2, H = 6·0; $C_6H_{10}O_5$ requires C = 44·4, H = 6·2. The substance was oxidised with nitric acid, no indications of saccharic, oxalic, or tartaric acids were given, but it yielded the pyrrol test for mucic acid. The substance probably belongs to that ill-defined family of vegetable carbohydrate gelatinoids, of which agar-agar is the best known member. The body is apparently identical with a similar substance described by Professor Stockman in his report.

Cygnose, $C_6H_{12}O_6$.—After the removal of the gastrolobin by means of lead acetate, the limpid solution gave tests with phenylhydrazine and Fehling's solution that pointed to the presence of a sugar in the solution. It was found quite impossible to obtain a pure sugar from the solution. The osazone was therefore prepared in the ordinary way. It can be crystallised from alcohol in the form of slender yellow needles, m. p. 179° C. The mean of four analyses gave C = 60·0, H = 6·3, N = 15·7. Calculated for $C_6H_{10}O_4(N_2H.C_6H_5)_2$, C = 60·3, H = 6·1, N = 15·6.

The sugar itself seems to be optically inactive both before and after inversion. It is not fermented by yeast.

In view of the presence of saponin, quercitrin, or allied bodies in plants of this group, reported by Professors Stockman and Dunstan, special search was instituted for these two bodies, but with negative results.

Toxic Action of Cygnine.—During the course of the research, the poisonous nature of the products obtained was from time to time established. With cygnine hydrochloride the following tests were made, with the assistance of Doctor Blackburne, Government Bacteriologist.

One-tenth of a grain of the hydrochloride dissolved in a few minims of water, and injected subcutaneously into a guinea-pig weighing 568 grammes, killed the animal in 7 minutes. One-twentieth of a grain killed another guinea-pig (348 grammes) in 22 minutes. Confirmatory evidence was also obtained by an experiment on a sheep. Seven grains of the salt in aqueous solution was injected into a sheep weighing 50 to 60 lbs. The first spasm was manifested 5 minutes after injection; in 6 minutes the animal fell to the ground in a rigid condition, and was subjected to renewed spasms up to 12 minutes after injection, when death ensued.

The following is an extract from Dr. Blackburne's notes on the first experiment:—

"After remaining quiet for 2 or 3 minutes it became rigid, with head stretched forward, then returned to normal, but in a minute or so rapid convulsions came on. The teeth could be heard to shut with a snap, the body became rigid, but there was no obvious opisthotonos. These spasms occurred three or four times in 7 minutes; the animal was quite dead. A post-mortem examination was immediately made. There was marked venous engorgement throughout the parenchymatous organs and the lungs, and all the large veins were much dilated. There was no local reaction at the point of injection. The heart was greatly overfilled, every chamber being much distended with dark blood. One hour and a-half afterwards, the heart (still *in situ*) responded to stimuli. Death was due evidently to asphyxia, probably secondary to convulsions. It would appear from this that the poison belongs to that group of which the principal one is strychnine."

Isolation of the Alkaloid from the "Box" Poison Plant, Oxylobium parviflorum.

Lobine, $C_{23}H_{31}N_3O_4$.

Experience having been gained in the examination of the "York Road" plant, the same kind of treatment was adopted in this case. The temperature throughout the whole investigation was kept as low as possible, never being allowed to rise above 35° C.

By this means a crystalline hydrochloride was obtained. This salt crystallised in needles, was very hygroscopic, and decomposed when its aqueous solution was heated above 35° C., a substance crystallising in cubes being formed; in spite of its hygroscopic character, it is but sparingly soluble in water (less than 1 per cent.). Its toxic action was shown by injection of a guinea-pig: one-tenth of a grain killed in five minutes. The symptoms were similar to those caused by cygnine.

The alkaloid itself was only obtained in a semi-crystalline condition by very careful spontaneous evaporation of the alcoholic solution in the dark; as a rule, it remained as an amorphous substance resembling isinglass. It decomposed on heating. An analysis gave the following results (the mean of four analyses):—C = 67·1, H = 7·7., N = 10·2. Calculated for $C_{23}H_{31}N_3O_4$, C = 66·8, H = 7·5, N = 10·2.

The gold salt was prepared as an amorphous yellow precipitate; it decomposes on exposure to light and moist air, and melts at about 100° C. It is freely soluble in alcohol, but sparingly so in water. On analysis, the mean of five analyses gave C = 36·3, H = 4·4, N = 5·5, Au = 26·1, Cl = 19·0. Calculated for $C_{23}H_{31}N_3O_4, HAuCl_4$, C = 36·7, H = 4·25, N = 5·6, Au = 26·1, Cl = 18·9. This new alkaloid has been named "*Lobine.*"

In nearly all its characteristics it resembles cygnine, but it is more stable, for the free alkaloid was isolated. Like cygnine, its hydrochloride, on heating, yields a non-nitrogenous substance, crystallising in cubes, and a nitrogenous uncrystallisable compound. These cubical crystals are fairly soluble in water, very soluble in alcohol, but insoluble in ether. They melt without decomposition at 107° C. They are non-nitrogenous and faintly acidic. The mean of two analyses was C = 63·3, H = 8·4. Calculated for $C_9H_{14}O_3$, C = 63·5, H = 8·2.

Isolation of Malic Acid from "Box" Poison Plant.

The lead precipitates obtained during the clearing operations in the isolation of the alkaloid were treated with sulphuretted hydrogen. From the filtrate an acid was obtained that gave a white amorphous precipitate with silver salts; a white, cloudy precipitate, heavy on boiling with calcium hydrate; and a precipitate with lead salts. The acid solution was neutralised with ammonia, treated with alcohol to precipitate mucilaginous matter, and evaporated to dryness. Deliquescent crystals resulted. These were then heated on a water-bath with excess of lime water, when a crop of crystals gradually deposited. The free acid prepared from this salt was very soluble in water. The mean of two analyses gave C = 35·8, H = 4·6. Calculated for $C_4H_6O_5$, C = 35·8, H = 4·5. From this and the properties of the acid it is probably malic acid. The calcium salt was also analysed, found

Ca = 20·9. Calculated for $(C_4H_5O_5)_2Ca.H_2O$, C = 21·0. The free acid melts at about 100° C.

Examination for Carbohydrates.—Sugars seemed to be absent. A small quantity, however, of a mucilaginous carbohydrate similar to gastrolobin was separated.

Owing to the reported presence of quercitrin and saponin bodies, by Dunstan and Stockman respectively, special search was made for these bodies, but with negative results.

Observations on the Life-history of Leucocytes. Part II.—On the Origin of the Granules.

By C. E. Walker, Assistant-Director of the Cancer Research Laboratories, University of Liverpool.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received August 2, 1906,—Read February 7, 1907.)

[Plate 5.]

The granules that are so frequently found in leucocytes* generally seem to lie scattered quite irregularly in the cytoplasm of the cell in which they occur. It is possible, in the case of the leucocytes found in the spleen, lymphatic glands, and the blood of mammalia, that there never is any ordered arrangement of the granules. In the bone-marrow, however, where leucocytes containing granules are often extremely numerous, a section of properly preserved material will show that the granules in a large proportion of these cells are arranged in a more or less definite manner. The granules in these are, as a rule, oval in shape, and are seen to lie in sequence close to each other, so that a line drawn through their long axes would appear as a thread or wire coiled up irregularly in the cytoplasm of the cell (fig. 1). Given this line connecting them, the granules would exactly simulate beads threaded on a wire bent into irregular curves, and put into a small spheroidal space. There are many gradations in the regularity of this arrangement of the granules. It varies from the mere suggestion of some of them having been strung together to very definite order, and the joining of several end to end (fig. 2).

There are, again, other cells in which a large number of the granules are joined together, forming in places a thick, deeply-staining thread, the axis of which is continuous with the axis of the strings of separate granules (fig. 3). In some such cells the separate granules and the thick thread appear to be about equal in amount and continuous with each other (fig. 4), while in others there are but very few granules, and the thread preponderates. From these it is possible to pass, by almost insensible gradations, to cells where there are no granules, but only a thick thread coiled up around the nucleus (fig. 6). In some of these cells it is practically impossible to distinguish the nucleus, so dense and darkly-staining is the coiled thread (fig. 5). So misleading are these figures that for several months I took them for, and drew them as, the spireme stages in the prophase of division in which the nuclear membrane had disappeared. It was only when I found nuclei in a resting condition existing in the cells which also contained this thread, that the present interpretation was arrived at. A careful examination of the cells in this stage, however, will frequently, though not always, demonstrate the presence of a resting nucleus. Where the nucleus cannot be seen, the reason is generally very obvious: the thread is so deeply stained with the basic stain, and is so closely coiled, that it entirely obscures the similarly stained nucleus. Moreover, while the thread stains very deeply, the nucleus, apparently, stains less readily than usual, thus adding to the probability of its being overlooked.

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* As in a previous communication ("Observations on the Life-history of Leucocytes," 'Roy. Soc. Proc.,' B, vol. 78, p. 53), the term "leucocyte" is used in the widest possible sense, and is intended to include all the wandering nucleated cells of the body.

From this stage it is possible to pass again by practically insensible gradations to cells where the coiled-up thread occupies a space slightly larger than (fig. 7), equal to, or smaller than the nucleus, until we arrive at some where it seems to be about the same size in proportion to the nucleus as is the archoplasm in the case of the spermatid.

Though it has not been possible as yet to trace the origin of this thread farther, it is strongly suggested that it arises in the archoplasm, which is often seen to be connected with it (fig. 9).

During the whole of its existence the thread stains very deeply, and always with a basic in preference to an acid stain. It seems to show best with saffranin and basic fuchsin. When it has entirely broken up, the granules formed from it still stain in the same manner; but as they begin to lose their regular arrangement, so they seem to lose their affinity for the basic stain. It appears, however, that this happens but slowly in all such cells, and probably in many the granules never stain with the acid dyes. In bone-marrow large areas are often encountered where over 30 per cent. of the cells contain granules staining with a basic dye. In other parts of the body the cells containing such granules are not present in such large proportions, and we meet with more in which the granules take the acid dye.

The thread, which subsequently breaks up to form the granules, has only been met with in the cells in the bone-marrow, and has not been observed in leucocytes anywhere else in the body.

It would seem, then, that the granules arise from a threadwork forming part of the archoplasm, and which is probably derived from it originally. This thread grows and eventually breaks up to form the granules. During the whole of its existence it stains with a basic stain, reaching the maximum in staining capacity shortly before it breaks up. The staining capacity of the nucleus during this period seems to vary inversely with that of the thread. There seems to be no appreciable difference between the material forming the thread and the chromatin in an actively dividing cell. There is, however, no evidence of its having been derived from the nucleus.

The position, from a cytological point of view, is much obscured by the current methods of dealing with leucocytes. It is extremely difficult to compare the results obtained by the methods used by cytologists with what has been observed in specimens that have been dried, heated, fixed with alcohol, or otherwise treated in such a way as to materially alter the appearance and inter-relationship of the parts of the cell. The methods commonly used, however, have proved to be of the greatest value clinically, and with regard to the staining reaction of the granules, which have so largely entered into the current classification of leucocytes, there is nothing incompatible with the observations here recorded.

It has been seen that the staining reaction in some, at any rate, of the granular cells changes from basic to acid. It is highly probable that this change may be hastened or retarded by influences external to the cell while it is still in the body. This probability is increased by the fact that the proportion of acid to basic staining granules is increased by so crude an influence as additional heat and dryness after the cells have been removed from the body.

The presence, therefore, of varying proportions of cells containing the so-called acidophile and basiphile granules in different diseases is just what one would expect, and is no argument against a common origin of both from the thread here described as occurring in some of the cells in the bone-marrow.*

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* The material used has been chiefly obtained from the guinea-pig and rat. The same fixatives were used as described in "Observations on the Life-history of Leucocytes" ('Roy. Soc. Proc.,' 1906). Attention is also drawn to the remarks upon preservation in the same paper, as smears or films of bone-marrow will not show the figures here described. No matter how carefully a smear or film be prepared, there must always be a grave risk that the cells will be partially dried, and the least approach to drying is fatal to the proper preservation of the cells.

I have to thank Professor Sherrington for his kind advice, which has been of the greatest value to me, particularly when beginning my researches with regard to leucocytes.

[*Addendum, July,* 1907.—I would take this opportunity of pointing out the relationship between certain structures which arise from true archoplasms. Among such structures are the archoplasmic vesicles found in the cells of the testis, which develop into the cephalic cap of the spermatozoon; the similar structures (Plimmer's Bodies) which appear in some of the cells in malignant growths; and the granules in leucocytes. Whether these phenomena are connected in some way with the Maiotic Phase is not clear, though something of the kind would seem to be suggested.

Since the above communication was presented, my attention has been redirected to a communication by Professor Mathews, of Chicago University, on "The Changes in Structure of the Pancreas Cell."* I give the following summary of the results obtained by Professor Mathews and myself.

Mathews.	*Walker.*
(1) Certain spiral twists or coils are found in the cytoplasm of the pancreas cells at certain periods. They probably arise from the nucleus.	An archoplasm, *i.e.*, an area in the cytoplasm showing a denser and more homogeneous structure than the rest, is found in certain leucocytes. This archoplasm does not arise from the nucleus.
(2) The threads, or some of them, subsequently collect in a mass, forming the "so-called nebenkerns of the pancreas."	A coiled up thread, arising in the archoplasm, may be observed in some leucocytes. The archoplasm in the leucocyte is something entirely different to the "nebenkern of the pancreas cell."
(3) These spiral twists or threads proceed to decompose.	The coiled archoplasmic thread proceeds to grow until it surrounds and obscures the nucleus. So far from any decomposition taking place, there is never any cessation in the growth of the thread until it has invaded the greater part of the cytoplasm.
(4) A stage apparently intervenes between the disappearance of the threads and the appearance of the granules in any given portion of the cytoplasm of the cell.	The thread breaks up directly into a number of short lengths, which subsequently acquire an oval, and, later, perhaps a spherical shape.
(5) It is probable that the "zymogen granules and the cytomitoplasm of the inner zone arise as the products of the decomposition of the threads."	The granules found in the leucocytes described arise by the direct breaking up into definite segments of the thread. In these cells there is no cytomitoplasm, and the thread from its first appearance never shows any sign of decomposition.

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* 'Journal of Morphology,' vol. 15, 1899, Supplement.

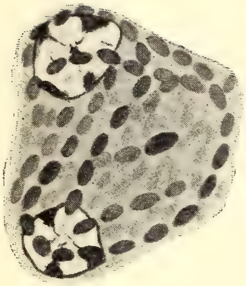
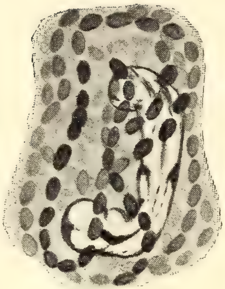


Fig. 1.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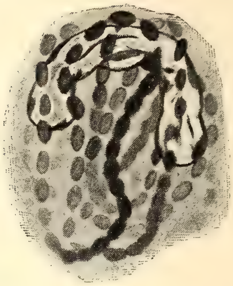
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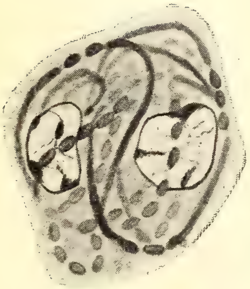
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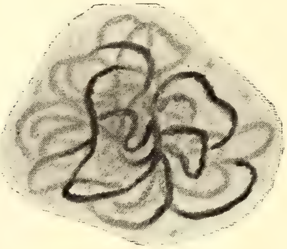
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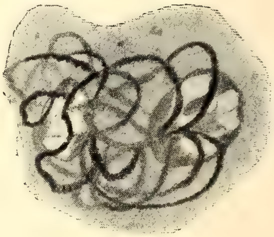
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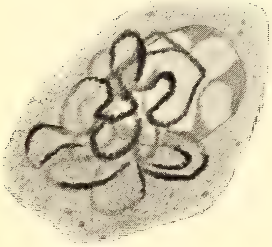
Fig. 7.

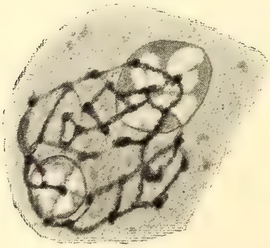
Fig. 8.

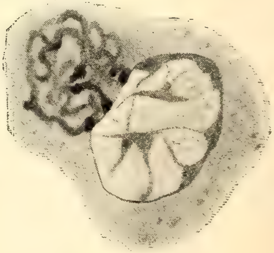
Fig. 9.

From the above it will be obvious that the second series cannot be regarded as any kind of confirmation of the first, for the sufficient reason that they possess nothing in common and deal with entirely different phenomena. For the same reason the two series do not contradict each other.]

DESCRIPTION OF PLATE.

(Drawn with a 2-mm. Zeiss apochromatic, 1·40 aperture, specially constructed for a 10-inch tube, and a 27 compens. ocular.)

All the cells illustrated are from the bone-marrow of the guinea-pig.

Fig. 1.—Cell showing the granules arranged more or less in strings.
„ 2.—The stringing of the granules is more marked, and some of them are joined end to end.
„ 3.—Still more of the granules are joined together, forming thick threads.
„ 4.—An earlier stage where granules and thread are about equal in quantity.
„ 5.—The thread before it has begun to break up. The nucleus is hidden.
„ 6.—The same stage with the nucleus showing.
„ 7.—An earlier stage. The nucleus here stains more faintly.
„ 8.—A slightly different form of thread, which is not uncommon.
„ 9.—A very early stage. The archoplasm is seen connected with the thread.

Observations on the Life-history of Leucocytes.—Part III.

By C. E. Walker, Assistant-Director, Cancer Research Laboratories,
University of Liverpool.

(Communicated by Professor J. B. Farmer, F.R.S. Received May 15,—Read June 27, 1907.)

[Plate 6.]

The phenomena here recorded were first observed among the leucocytes of Axolotl, which had collected in a mass owing to the presence of a foreign body or to the infliction of a slight wound.*

Some among the mass of leucocytes thus obtained were seen to be sending out protrusions from their nuclei. Often where this occurred the action seemed to be mutual on the part of two contiguous leucocytes (fig. 1). These protrusions were quite different in character from the processes which are sent out by the nuclei in polymorphonuclear leucocytes and myeloplaxes, and from the appearances presented during the phenomenon of amitosis.

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* The methods used for obtaining leucocytes apart from the other cells of the body were adapted from those of other observers who have investigated inflammatory processes. Small celluloid tubes were introduced under the skin of the animal and removed after being left in the body for varying periods of time. The tubes and their contents were, after removal, dropped into a suitable fixative and sections made in the usual manner. Better and more uniform results were, however, obtained by fixing portions of the coagulated exudation from a small scratch upon the skin of the animal.

Shortly after the nuclear protrusions had reached the periphery of the cytoplasm, which was always reached at adjacent points of the contiguous cells, the ends of the protrusions joined.* After the joining of these two processes, a ring of chromatin masses appeared at either end, a short distance outside the points where the peripheries of the respective nuclei of the two cells would have been if no protrusions had been formed (fig. 2). Strands of chromatin extended from these masses just under the membrane of both nuclei, and also along the tube which joined them. Other strands seemed to pass along the tube between the two nuclei. After this apparatus had been fully formed, the chromatin in one of the two nuclei began to disappear, and the amount of chromatin in the other was frequently very markedly increased. This process continued until one nucleus was completely denuded of its chromatin, and the nuclear membrane collapsed (figs. 2 and 3). The chromatin ring at the end of the tube contiguous to the collapsing nucleus remained long after the other had disappeared, and sometimes the termination of the tube and the ring appeared in a shape something like an acorn on a stalk (fig. 3). While this process seemed to be quite constant in so far as the disappearance of the chromatin from one of the nuclei was concerned, the increase of the chromatin contained in the other nucleus was not always very apparent. Frequently, however, there was no doubt that a very large increase indeed had occurred.

The leucocytes of Axolotl upon which these observations were made were, of course, in a more or less abnormal condition, but in view of the relatively small size of mammalian cells when compared with those of Axolotl,† it appeared likely that what was very striking in the case of the large cell might easily be missed in the case of the small. Some slides of normal mammalian tissues (bone-marrow, spleen, lymphatic gland, etc.) were re-examined, and all the stages in the phenomenon described above, excepting the first, were found in the normal mammalian spleen (figs. 4—8). In addition, an apparently later stage was found, where the collapsed nuclear membrane had almost disappeared, leaving only a few shreds (fig. 9). Hitherto this phenomenon has not been observed in any other mammalian tissue than normal spleen. It is probable, had the series of observations upon what happened among the leucocytes of Axolotl been carried further, that the later stage observed in the mammalian spleen would have been found. One would also expect to find the same phenomenon taking place among the leucocytes gathered together in the mammalian body under certain abnormal conditions.

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* It can but rarely happen that two contiguous cells will be in such a position in the section that the mutual sending out of nuclear processes will be presented to the observer in profile, as shown in fig. 1. Much more often either only one of the two cells will be found in the same section, or the two will be super-imposed either directly or at an angle. The phenomenon is therefore probably more common than would at first sight appear, as anything but a lateral presentation of the two cells would be difficult, often impossible, to interpret.

† Compare figs. 1—3 with figs. 4—9, which give a fairly accurate idea of the relative sizes of these cells.

There are some points of difference between the appearance of the figures in Axolotl and those in mammalian spleen. The ring of chromatin masses in the latter class of cells does not appear to be constant, or even frequent, at that end of the tube nearest the nucleus which is destined to absorb the other. Again, the evidence that the process is at first mutual, is questionable in the case of mammalian leucocytes, as no undoubted cases (such as shown in fig. 1) have hitherto been discovered. It may be, therefore, that the process commences mutually between two cells, one eventually gaining the upper hand in the case of Axolotl, while in mammals the process may never be mutual at any stage; but one cell always plays an active part, the other remaining passive. No other material points of difference have yet been observed.

In both Axolotl and mammalian spleen, however, there is an immediate conclusion with regard to these observations that appears quite clear. The nuclear contents, at any rate the chromatin, and probably the linin, of the one cell are directly absorbed by the other. The probability that the linin as well as the chromatin is absorbed is very great. The chromatin masses forming the rings at either end of the tube, and the strands of chromatin extending from these masses, are so sharply defined that it would seem that something must be holding the chromatin together in a definite form. The equally sharply defined strands of chromatin that pass through the rings appear to be continuous with the chromatin and linin of the nucleus.

The process of absorption begins by the mutual throwing out of nuclear protrusions of a peculiar form by two contiguous leucocytes in the case of Axolotl, while in the mammalian spleen one leucocyte apparently sends out a protrusion from its nucleus, which attaches itself to the nucleus of another. In either case the obvious result is: (1) one cell with a nucleus containing a double complement of chromatin, and probably of linin, and (2) a mass of protoplasm without a nucleus, which represents what was originally the other cell.

The mass of protoplasm which is left without a nucleus may be dismissed in a few words. It closely resembles what has been described elsewhere as the red blood corpuscle after it has lost its nucleus, and before it has become differentiated to its concavo-convex shape.* Whether some red corpuscles arise in this way must remain doubtful at present. There is, however, nothing against this being the case in the observations referred to, as red corpuscles seem to arise in the spleen, as well as in the bone-marrow.

The case of the leucocyte with a nucleus containing a double complement of chromatin and linin, however, calls for a careful consideration.

In a previous communication upon the life-history of leucocytes, observations were recorded which seemed to show that some, at any rate, of the leucocytes in the bone-marrow passed through the Meiotic Phase, and, after the number of the chromosomes had thus been reduced to one-half the normal somatic number, many post-meiotic generations occurred both in the bone-marrow and in the lymphatic glands, in which the reduced number of chromosomes was retained.† A comparison was made between these phenomena occurring among leucocytes and what happens in the case of many plants. At the same time, it was suggested that, while the large number of post-meiotic generations, and the fact that but few of the cells of these generations were destined to conjugate with other reduced cells in the case of plants, offered a parallel with what apparently happened in the case of leucocytes, an important difference existed, in that there was no evidence that any among the post-meiotic generations of leucocytes ever reached a stage where they conjugated with other reduced cells.

The observations recorded in the present communication suggest that this comparison may now be carried much further, not only between certain phenomena exhibited by plants and leucocytes, but also between those exhibited by plants and unicellular organisms on the one hand and leucocytes on the other. This further comparison depends upon the interpretation placed upon the absorption of the nucleus of one leucocyte by another, and is only possible if this is regarded as a process of fertilisation.

There is a considerable amount of evidence that these leucocytes in which the fusion of nuclei takes place have passed through the Meiotic Phase. The members of this particular class of leucocytes are frequently found to be dividing mitotically, very commonly exhibiting the reduced number of chromosomes.

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* C. E. Walker, "On the Origin and Differentiation of the Red Blood Corpuscles in Mammals," 'Trans. Path. Soc. London,' vol. 58, Part I, 1907.

† C. E. Walker, "Observations on the Life-history of Leucocytes," 'Roy. Soc. Proc.,' B, vol. 78, 1906.

This class of leucocytes, which is described in more detail elsewhere,* forms a large proportion of the cells in the spleen and the majority of those in the lymphatic glands.

It is necessary, before considering the probability of fertilisation being a correct interpretation, to dismiss the probability of the process being simply the destruction of one cell by another. This is a very common process, but seems always to take the form of engulphment of the whole or part of the cell destined to destruction into the cytoplasm of another cell. The usual sequence in the case of vertebrates is, that one leucocyte or other kind of cell engulphs another bodily into its cytoplasm. The engulphed cell is gradually disintegrated. The nucleus first shows signs of degeneration. At a later stage the whole cell somewhat resembles an archoplasmic vesicle or Plimmer's body, and eventually it disappears altogether, both nucleus and cytoplasm. The engulphed cell is never taken into the nucleus, but remains in the cytoplasm of the engulphing cell, and is there apparently digested. This phenomenon may be seen taking place in many different tissues, particularly where there is any disturbance from the normal condition. It is so totally unlike what has been here described as taking place among the leucocytes in Axolotl and mammalian spleen, that it is unnecessary to go into further details.† The process of engulphment and destruction of one cell by another is, in fact, morphologically a very simple and straightforward affair, and takes place without the development of any special apparatus on the part of either. On the other hand, the absorption of one leucocyte nucleus by another, as here described, seems to involve the development of a special and complicated apparatus, formed in such a manner that the chromatin and linin of one nucleus may be transferred directly to the other, without any process of digestion by the cytoplasm being possible.

It might possibly be suggested that certain prolongations of the nucleus and cytoplasm which are very common among the leucocytes found in the mammalian spleen might be confounded with the figures here described. A careful examination shows that they are of quite a different nature, and so dissimilar as to be hardly comparable.

The morphological evidence would therefore appear to be in favour of fertilisation, in that the nuclear contents of two cells are apparently fused without any disintegration of the contents of either nucleus taking place, and that the fused nuclear contents continue to exist in a common cytoplasm.

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* C. E. Walker, "On the Origin and Differentiation of the Red Blood Corpuscles of Mammals," *loc. cit.* *Ibid.*, "Observations on the Life-history of Leucocytes," *loc. cit.*

† Figures illustrating the appearance of engulphed cells may be seen in the 'First Report on the Cytological Investigation of Cancer,' Moore and Walker, 1906, Cancer Research Laboratories, University of Liverpool.

If we accept the interpretation that the apparent fusion of the nuclear contents of two leucocytes is to be regarded as the fertilisation of the one by the other, a remarkably complete, although complex series of phenomena is suggested; a series of phenomena which, however surprising it may appear at first, is found on careful examination to be analogous in many respects, even in detail, to several equally complicated series of phenomena that have been generally acknowledged to occur.

It is necessary here to describe briefly those phenomena in plants with which it is proposed to compare those occurring among leucocytes.

A certain group of cells belonging to the soma becomes differentiated and passes out of somatic co-ordination. Some of these, the tapetal cells, without passing through the Meiotic Phase, exhibit changes which render them different in function to the cells of the soma. They are destined to disintegration in a comparatively short time, quite independently of what happens in the soma or among the rest of the cells of this differentiated group. They serve a nutritive purpose. Other cells of the group pass through the Meiotic Phase. Of these, only a few of the individuals produced by many subsequent generations are destined to reach the stage where conjugation may take place with other cells in the same condition. The rest produce a comparatively unlimited number of post-meiotic generations, and carry out the function of nourishing and supporting those cells destined to conjugate.

Apparently a number of the cells belonging to the early generations of leucocytes do not pass through the Meiotic Phase, but though they are apparently out of co-ordination with the soma, they serve nutritive and other purposes, some among them developing granules.*

Other leucocytes, apparently belonging to later generations, pass through the Meiotic Phase. Of this group the greater number appear to be converted into red corpuscles, thus serving a nutritive purpose.† Others of this group, if the fusion of nuclear contents be regarded as fertilisation, conjugate with other reduced cells.

It may be that when, during the process of evolution, leucocytes or their immediate precursors were developed and passed out of co-ordination with the parent soma, these cells reverted to the characters of a remote protozoan ancestor. This phylogenetic reversion of certain cells produced by the soma may have become constant, and new characters may have been developed from this fresh point of departure. This would account for the phenomena exhibited by the leucocytes in the vertebrates. It has been considered probable that spermatozoa are also examples of phylogenetic reversion to the characters of protozoan ancestors.

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* C. E. Walker, "Observations on the Life-history of Leucocytes, Part II," *supra*.

† C. E. Walker, "On the Origin and Differentiation of the Red Blood Corpuscles in Mammals," *loc. cit.*

The possible conclusions to be derived from these observations may be briefly stated as follows: Certain cells, probably only existing in the bone-marrow in the case of the adult mammal, give rise to a group of cells which are, or will immediately pass, out of somatic co-ordination. Some of these are destined to comparatively rapid disintegration, probably including the nutrition of other cells among their functions. Others pass through the Meiotic Phase, and continue to divide, producing an apparently unlimited number of post-meiotic generations of cells possessing half the somatic number of chromosomes. The great majority of these are converted into red corpuscles, serving a nutritive purpose. The remainder are fertilised in the manner described in the observations here recorded, and, as is the case in many unicellular animals, after fertilisation divide a number of times, thus producing fresh generations of leucocytes, which may or may not subsequently pass through the Meiotic Phase again.

This would naturally lead to the further conclusion that the leucocytes passing out of co-ordination with the soma live as parasites upon the parent organism, and in themselves possess a complete life cycle.

These observations would appear to have some bearing upon what has been described as occurring in malignant growths. It has been stated that during the early stages of malignant growths, leucocytes, entering into the cytoplasm of some of the tissue cells, proceed to divide mitotically, synchronously with the nucleus of the cell that they have invaded, the chromosomes of the leucocyte and the tissue cell becoming mixed and being distributed between the daughter nuclei resulting from the mitosis.* This bastard form of fertilisation seems to suggest some properties in the leucocytes different to those possessed by any other cells in the body, excepting perhaps the sexual cells. This suggestion is strengthened by the observations here recorded.

A fusion between the cells of certain graftable tumours in mice has been described.† These tumours were said to be malignant, though some doubt has since been thrown upon this statement. A re-examination of the figures illustrating this alleged fusion of cancer cells of mice suggests very strongly that some of them may be fusions of leucocytes such as are here described, and that the observers were mistaken in supposing that the cells in question were cancer cells. That a large congregation of leucocytes does occur in the case of Jensen's graftable tumour renders this extremely probable, for we have seen that among the leucocytes brought together by the introduction of a foreign body in the case of Axolotl, some of them conjugated. Moreover, nothing in the nature of a conjugation between cancer cells has been recorded by any other observers, although the alleged fusion has been diligently sought for during several years.

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* Farmer, Moore, and Walker, "On the Behaviour of Leucocytes in Malignant Growths," 'Trans. Path. Soc. London,' vol. 56, Part III, 1905. *Ibid.*, "On the Cytology of Malignant Growths," 'Roy. Soc. Proc.,' B, vol. 77, 1906. Moore and Walker, 'First Report on the Cytological Investigation of Cancer,' University of Liverpool, Cancer Research Laboratories, 1906.

† Bashford and Murray, 'Roy. Soc. Proc.,' 1904.

DESCRIPTION OF PLATE.

Fig. 1.—Two leucocytes in Axolotl in which the nuclei are sending out protrusions.

Fig. 2.—The two nuclear protrusions have joined, rings of chromatin masses have appeared at either end of the tube thus formed, and the chromatin is disappearing from the lower of the two nuclei.

Fig. 3.—The nuclear membrane of the cell on the right is collapsing. The ring of chromatin masses has disappeared at the end of the tube nearest the nucleus that has absorbed the chromatin of the other.

Fig. 4.—Two leucocytes from the spleen of the guinea-pig, showing the nuclear protrusion from one attaching itself to the nucleus of the other.

Fig. 5.—A slightly later stage.

Figs. 6, 7, and 8.—Stages showing the absorption of the contents of one nucleus by the other.

Fig. 9.—Showing the complete collapse of the nuclear membrane of one leucocyte, with only a few shreds of the membrane remaining.

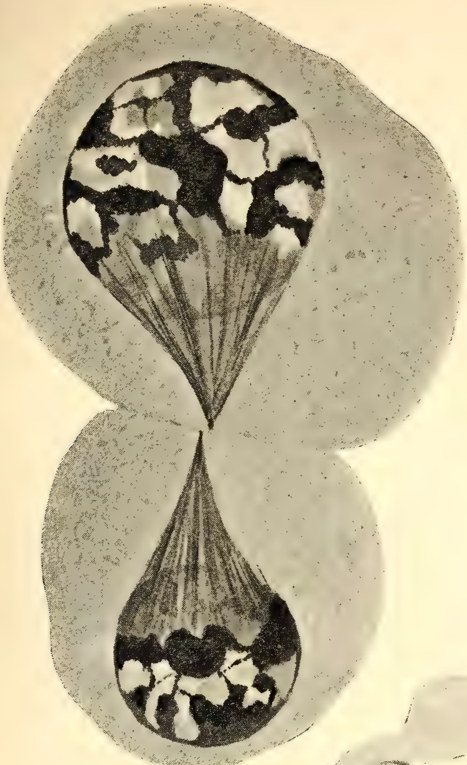

Fig. 1.

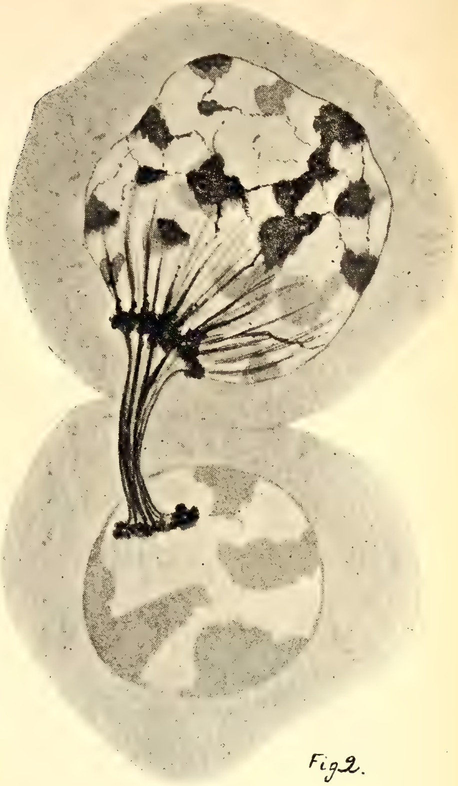

Fig 2.

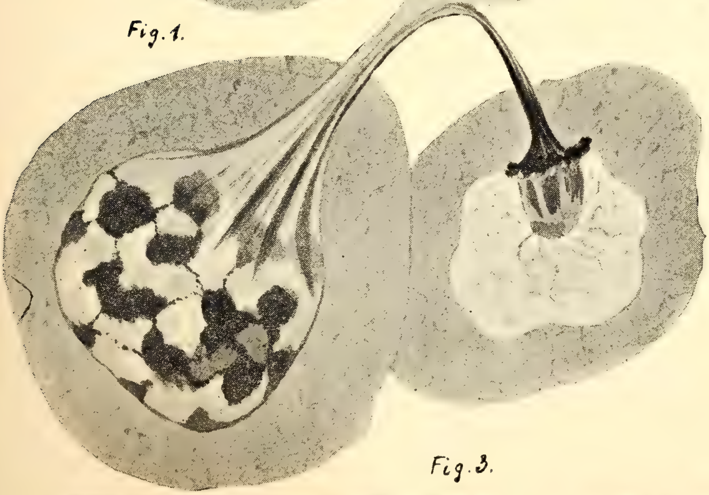

Fig. 3.

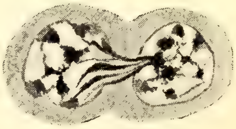

Fig. 4.

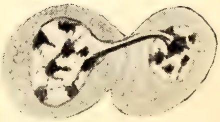

Fig. 5.

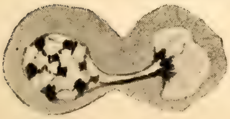

Fig. 6

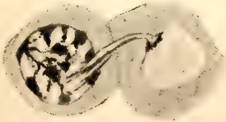

Fig. 7.

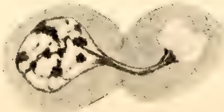

Fig. 8.

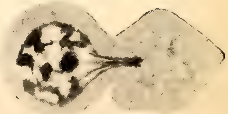

Fig. 9.

C.E. Walker del.

The Time taken in passing the Synapse in the Spinal Cord of the Frog.

By Florence Buchanan, D.Sc., Fellow of University College, London.

(Communicated by Professor Sherrington, LL.D., F.R.S. Received March 1, 1907.)

(Abstract.)*

The time taken by an impulse to cross a single synapse has been estimated by the measurement and comparison of cord-delays in different reflexes of the decerebrate frog. The cord-delay was determined by measuring, (1) the interval of time which elapsed between the moment of application of a single break induction shock to a nerve containing afferent (sensory) fibres and the moment of appearance in a particular spot of a particular muscle of a response, known to have no latency of its own (the electrical response), and shown to have been produced by the excitation of the afferent fibres; by then measuring and deducting from this time-interval, (2) that which elapsed before a response occurred in the same spot of muscle when the same or a similar induction shock was applied to the efferent (motor) fibres of the nerve supplying it. From the remainder the time was deducted which would be taken by an impulse travelling at the rate of 30 metres per second to traverse the measured length of nerve between the two points of excitation.

The recording of the intervals was automatic, the movements of the meniscus of the capillary electrometer, the two terminals of which were connected with the muscle, being photographed on plates moving at known rates. The recording muscle chosen was the gastrocnemius, and the nerves stimulated were the sciatics of the same and of the opposite side. The experiments were all made on autumn or winter frogs.

It was found that a single induction shock was only capable of producing a true reflex response in the gastrocnemius from a normal cord when applied to the sciatic of the same side. The same stimulus applied to the opposite sciatic only produced the response when the excitability of the cord had been raised by some drug. The drugs used for the purpose were strychnine and phenol.

When the same-side sciatic was stimulated, the reflex response in the muscle was quite distinct from the response produced directly by the excita-

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* The paper will be published in full in the first number of the Physiological Part of what was the 'Journal of Anatomy and Physiology,' to appear in January, 1908, edited by Professor Schäfer, F.R.S.

tion of the motor fibres. The difference of time between the two responses was hardly altered by the administration of strychnine so long as the dose was not strong enough to produce spasms or to affect the general circulation. It was, however, slightly shortened, the cord-delay varying in normal cords between 0·012 and 0·022 second, and in strychnine cords between 0·009 and 0·020 second. With stronger doses of strychnine the cord-delay was considerably lengthened, probably as the result of impairment of the circulation.

When the sciatic of the opposite side was stimulated and the excitability of the cord raised just sufficiently for a reflex effect at all to be produced by the stimulus, the cord-delay was always about double what it was in the same-side reflex. In the strychnine cord of preparations the circulation of which was still unimpaired, the extra-cord-delay in the case of the crossed reflex became very rapidly shorter as the action of the drug continued and might even be reduced to one-fifth of its original value in a preparation in which the total cord-delay in the same-side reflex was hardly altered during the experiment. It was, however, never reduced to less than 0·004 second. In preparations in which the reflex responses were spasms, or in which the circulation was impaired, it also was always long, although not usually quite so long as the total cord-delay in the same-side reflex. In such preparations in both kinds of reflexes the central stimulus often failed to produce its maximum effect on the muscle at the beginning of the response.

The effects of altering the temperature of the cord, and of altering the strength of the artificial stimulus applied to the nerve, and of fatigue, on cord-delay and on extra delay respectively in the two kinds of reflexes are dealt with in the full paper.

It is suggested that the method of measuring cord-delay employed gives the clue required for ascertaining the number of synapses involved in succession in any particular reflex, and that there is normally *one* synapse interposed in the conductive path of each individual fibre concerned in the same-side reflex investigated, *two* in that of each fibre concerned in the crossed reflex. It is pointed out that the numerical results obtained refer, as a rule, rather to the modal time than to the shortest time in which a synapse may be passed, and reasons are given for believing that the set of synapses alone involved in the same-limb reflex, the primary synapses, are wont to act much more synchronously than the set of secondary synapses involved, in addition, in the crossed reflex, when the cord is normal. The nature of the action of strychnine on the cord is also discussed.

The experiments were made in Oxford, in the autumn of 1906, with the aid of the Government Grant administered by the Royal Society.

A Preliminary Summary of the Results of the Experimental Treatment of Trypanosomiasis in Rats.

By H. G. Plimmer, F.L.S., and J. D. Thomson, M.B., C.M.

(Communicated by Sir Ray Lankester, K.C.B., F.R.S. Received July 20, 1907.)

The experiments of which the following note is an abstract have been undertaken under the direction of the Tropical Diseases Committee of the Royal Society.

The strains of trypanosomes which have been used in these experiments are a Nagana from the original strain brought to England, and a Surra from Professor Lingard in India. The Nagana strain kills rats in an average time of 5·5 days, and the Surra strain in 6·9 days.

Chinolin Compounds.

The knowledge of the action of quinine in malaria suggested the trial of various chinolin compounds. As is well known, quinine itself has no influence in trypanosomiasis. Cyanin was the substance first tried, as this in extremely dilute solutions (1—10,000) is very poisonous to trypanosomes outside the body. A series of Nagana and Surra rats were given doses of from ½ to 1 milligramme (which is a poisonous dose for small rats), but all of these died about the same time as the controls, and in every case the trypanosomes had increased in the usual manner, so that at death the blood was as swarming with trypanosomes as if no treatment had been used. Messrs. Bayer and Co., of Elberfeld, kindly placed at our disposal nine chinolin and chinaldin compounds and six "cyanin farbstoffe," but none of these produced any effect on the development or course of the diseases.

Dichlorobenzidine + Acid H.

This substance, which was first used by Nicolle in trypanosomiasis of mice, was found, when used both on mice and rats, to lengthen the course of the diseases, but in no case was the extension of the course of the disease very marked. Nagana mice, after one dose of 1 c.c. of a 1-per-cent. solution, lived from 8 to 11 days, and Surra mice from 10 to 16 days, one living as long as 29 days; in Nagana rats the disease was prolonged to an average time of 14 days, and in Surra to an average of 16 days. In all the animals treated with this substance the spleen and liver were very much enlarged, and living trypanosomes were found *post mortem*, showing that the substance had not practically influenced the pathological processes. In no case was the substance completely absorbed.

Trypanroth.

This was discovered and first used by Ehrlich. When used alone it lengthened the course of the disease in both Nagana and Surra to about 14 days, but in all cases the spleen was found to be as much enlarged as in the control, and living trypanosomes were invariably found *post mortem.*

Arsenious Acid.

This was used at first simply dissolved in water, and later, in order to try to avoid the irritation and sloughing invariably produced, in a solution slightly over-neutralised with carbonate of soda. When injected before the fourth day of Nagana and Surra, arsenious acid caused the disappearance of the trypanosomes from the blood, but they invariably recurred before death. It was given in doses of 1 milligramme, and one rat lived as long as 26 days, two to 16 days, and one to 17 days, each of these rats having taken 6 milligrammes altogether. A very considerable effect was produced on the vitality of the trypanosomes, as a rat inoculated from the bone-marrow of one of the 16-day rats did not show trypanosomes in the blood until the ninth day after inoculation. Whether neutralised or not, sores were produced at the points of injection. The spleen was found *post mortem* to be about as large as that of the controls.

Atoxyl.

This substance, which was first used by Thomas and Breinl, has been found by Ehrlich and Bertheim to be the sodium salt of para-amido-phenyl-arsenic acid, with the formula

$$NH_2(C_6H_4)AsO.ONa.OH,3H_2O,$$

and Moore, Nierenstein, and Todd* have confirmed this view of its composition, which differs from that originally published, which stated it to be an anilide of metarsenious acid.

This is the most important substance, so far discovered, in relation to the treatment of trypanosomiasis. In all cases of Nagana and Surra (with the exception of the atoxyl-proof cases mentioned below), atoxyl causes the entire disappearance of the trypanosomes from the blood, so that rats inoculated with the blood when it was microscopically free from parasites failed to take the disease; but the trypanosomes, in our experience, have invariably recurred, and death was only delayed for a period varying with the dose and with the time of commencement of the treatment. When the dose was sufficient, *e.g.*, 1 to 1·5 c.c. of a 5-per-cent. solution in three to five doses

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* "On the Treatment of Trypanosomiasis by Atoxyl . . . . followed by a Mercuric Salt," etc., 'Biochemical Journal,' vol. 2, Nos. 5 and 6.

(a dose being given every, or every other, day), even after as many as four recurrences the spleen was generally found *post mortem* to be very little or at most moderately enlarged. A too large dose apparently produced wasting, and a bad condition of the hair, and, we think, a quicker return of the trypanosomes.

When atoxyl is given more continuously or too freely than is required, in cases in which there have been many recurrences, and probably under some other conditions of which we are ignorant, in a certain small proportion of rats so treated a race of trypanosomes is produced which entirely resists atoxyl, and continues to develop and multiply in spite of continued exhibition of the drug. This strain, when inoculated into fresh rats, retains its resistance to atoxyl. Ehrlich, who has produced such a strain in mice, calls them "atoxyl-fest," and we have obtained this atoxyl-proof variety of trypanosome in rats, both in Nagana and Surra, of which mention will be made later.

1. *Monophenylarsenic acid.*
2. *Nitrophenylarsenic acid.*
3. *Paratolylarsenic acid.*

These three bodies were sent for experimental purposes to Professor Cushny, F.R.S., by Professor Michaelis, who discovered them. They are of interest in connection with the treatment of trypanosomiasis, since atoxyl is an amido derivative of phenylarsenic acid, which was also discovered by Michaelis. They are extremely poisonous substances, and were given in doses of 1 c.c. of a solution of 1/400.

1. Monophenylarsenic acid.—This, in common with other arsenic compounds, notably affected the development of the trypanosomes. In doses of 1 c.c. of 1/400, repeated once or twice in untreated animals, it diminished greatly the number of trypanosomes; and in three recurrent cases, after initial treatment with atoxyl, it caused the trypanosomes to disappear, but it did not save any animal; and it is, as are all these three compounds, poisonous in effective doses.

2. Nitrophenylarsenic acid.—In untreated animals, and in two recurrent cases after atoxyl, this caused a temporary disappearance of the trypanosomes; in a case of second recurrence after atoxyl, it produced no effect on the trypanosomes. It is the most poisonous of the three.

3. Paratolylarsenic acid.—This caused a more effective disappearance of the trypanosomes than the other two compounds, and it was better borne. One Nagana rat, treated only with it from the beginning, lived for 25 days, having had, however, three recurrences during this period. At each recurrence the trypanosomes disappeared after two doses of 1 c.c. of 1/400. In recurrent

cases after atoxyl a temporary disappearance of the trypanosomes was also effected.

Other Arsenic Compounds.

Arrhenal (di-methyl sodium arseniate) and sodium cacodylate were also tried, but were found to have no effect whatever on the course of the disease, nor upon the development of the trypanosomes either in first infections or in recurrences.

Other Bodies.

Fluorescein was tried without any effect; and also sodium cinnamate, on account of its reputed property of producing leucocytosis, which might have been useful from the phagocytic standpoint. But it failed to have any effect in these diseases.

Treatment with two or more Drugs.

The deduction from the above-mentioned experiments with single bodies is that, at the present time, there is no substance known which alone will cause a permanent disappearance of the trypanosomes from the affected animal, *i.e.*, effect a cure; so that experiments have been carried out to see whether any combination of bodies would produce the desired effect. The compounds of mercury seemed from their known properties to be the most promising, and the results of experiments in this direction, lately published by Drs. Moore, Nierenstein, and Todd, are most encouraging. We have not used the form of mercury (liq. hydrarg. perchlor.) with which these good results have been obtained, but we have used the lactate, sozoiodol, and succinimide, and also Donovan's Solution (liq. arsen. et hydrarg. iodid.). Of these the succinimide appears to us to be the best, as it is unirritating, and will apparently mix with atoxyl without change—at any rate it does not interfere with the action of the atoxyl; the lactate is very irritating, even in doses of one-third of a minim of the ordinary hypodermic solution, and it is not so effective as the succinimide; the sozoiodol appears to come between these two in point of efficiency. But in our experience there is a great difference in the individual equation of rats with regard to dosage and poisonous effects, which the consideration of relative weight does not help us to gauge, so that there has been difficulty in ensuring the best method of giving these drugs, and of determining their appropriate quantity. The duration of the disease has been very greatly prolonged in most cases, and in some we have confidence that a cure has been effected.

Of all the substances tried, atoxyl has had by far the most favourable action, and it is easily tolerated, and produces no irritation. But, as mentioned before, it has no permanent effect; it causes the disappearance of the trypanosomes from the blood, even when in very large numbers, but there remain behind certain forms of the parasites which can resist it, and which after an interval can reproduce the general infection with the indifferent forms. These recurrences can take place, in our experience, up to as many as seven times. The resistant forms appear to be located in the intermediate periods in the bone-marrow, but there is some doubt about this yet, as in some cases we have failed to produce an infection, even with a quantity of the marrow. It is probable also that some of these resistant forms may be found in the glands, and observations are being carried out on these points. But when an infection is produced from the marrow of these atoxyl-treated rats, the incubation period is very much prolonged, and it may be as long as nine days before trypanosomes can be found in the blood of the inoculated animals.

Atoxyl appears to act not by virtue of the arsenic in it alone, but its effects are probably due to this in combination with some other of its constituents, as its action is much more rapid and complete than that of any other of the simpler arsenic compounds which we have tried.

The following Tables I and II give the results of treatment with atoxyl and succinimide of mercury: this latter has been given either with the atoxyl or immediately after it, the best method being, we think, to give two or three doses of atoxyl and to give a dose of succinimide of mercury at the same time as the second and third doses, with perhaps one after. Some of our rats have had too much, and we are now giving less with better effect. The succinimide has the great advantage of being unirritating to the tissues, and it will mix with atoxyl without precipitation, and without, at any rate, interfering with the action of the latter.

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury. Average duration of untreated disease, 5·5 days.

No.	Weight.	Quantity of 5 p. c. atoxyl.	Quantity of succinimide of mercury.	Recurrences.	Lived.	
	grammes.	c.c.	milligrammes.		days.	
1	175	2·4	1·5	2	23	Had young on 20th day.
2	210	2·85	1·75	1	30	Did not die of disease.
3	250	2·1	2·5	1	28	Kidneys degenerated.
4	200	2·1	2·75	0	—	*Living* 143 *days.*
5	175	2·0	0·75	1	27	Did not die of disease.
6	150	1·8	1·25	0	—	*Living* 140 *days.*
7	200	1·8	1·25	0	—	*Living* 136 *days.*
8	150	2·75	2·0	6	79	
9	175	2·6	1·25	0	22	Succinimide given too long after the atoxyl.
10	175	3·0	1·25	1	—	*Living* 92 *days.*
11	125	1·8	0·8	0	38	
12	200	1·2	0·75	1	—	*Living* 85 *days.*
13	160	2·8	0·75	3	36	
14	210	4·5	1·75	4	59	
15	175	1·75	0·5	0	—	*Living* 78 *days.*
16	190	1·4	0·25	0	—	*Living* 75 *days.*
17	200	4·1	1·75	4	42	Trypanosomes plentiful at time of death, probably atoxyl-proof.
18	175	6·35	2·0	7	107	This rat had 5 minims. of Donovan's solution on the initial appearance of trypanosomes, but, as it had no effect, atoxyl, and subsequently succinimide, were given.
19	160	4·1	0·75	3	61	
20	150	3·7	0·5	3	44	Given paratolylarsenic acid after third recurrence, which killed the rat.
21	150	0·7	0·75	0	—	*Living* 37 *days.*

Table II.—Surra Rats treated with Atoxyl and Succinimide of Mercury. Average duration of untreated disease, 6·9 days.

No.	Weight.	Quantity of 5 p. c. atoxyl.	Quantity of succinimide of mercury.	Recurrences.	Lived.	
	grammes.	c.c.	milligrammes.		days.	
1	175	1·5	2·25	0	43	Did not die of disease.
2	175	1·5	2·25	0	68	Did not die of disease.
3	150	1·5	1·25	0	44	Did not die of disease.
4	200	5·1	2·75	3	63	
5	125	1·8	1·5	0	—	*Living* 137 *days.*
6	125	1·8	0·5	0	120	Did not die of disease.
7	155	1·8	0·75	1	24	
8	175	2·45	1·0	1	23	This quantity of succinimide produced an acute nephritis in this rat, with necrosis of the epithelium of the convoluted tubes, and many hæmorrhages.
9	160	3·3	1·0	1	—	*Living* 91 *days.*
10	150	3·0	0·75	4	55	
11	150	2·4	0·75	2	32	
12	160	2·1	0·37	1	22	Treatment suspended on 10th day.
13	160	1·8	0·25	2	32	Did not die of disease.
14	145	2·1	0·75	0	—	*Living* 42 *days.*
15	120	1·9	0·5	0	—	*Living* 39 *days.*
16	125	3·0	1·0	2	34	This rat had 2 c.c. iodipin on 3rd day.
17	200	4·1	1·75	4	42	
18	145	1·05	0·25	0	—	*Living* 42 *days.*
19	120	0·95	0·5	0	—	*Living* 39 *days.*
20	145	1·05	0·65	0	—	*Living* 33 *days.*
21	125	2·1	0·75	3	54	This rat had at the outset 8 mgms. in all of arsenious acid, the atoxyl being given on the first and subsequent recurrences.
22	155	1·5	1·0	1	32	This rat had also initial treatment with arsenious acid, of which it had 6 mgms. in all, the atoxyl being given on the first recurrence. The rat did not die of the disease, but from injury during pregnancy.

(See also No. 8, Table III.)

Table III gives the results of treatment with atoxyl and mercury sozoiodol, from which it will be seen that the results are not so good as those with succinimide of mercury. Mercury sozoiodol will not mix with atoxyl, a dense precipitate being formed when the solutions are mixed. The solution used was the ordinary hypodermic solution (1·5 per cent.) prepared by Martindale.

Table III.—Nagana and Surra Rats treated with Atoxyl and Mercury Sozoiodol. Average duration of untreated diseases 5·5 and 6·9 days respectively.

Disease.	No.	Weight.	Quantity of 5 p. c. atoxyl.	Quantity of mercury sozoiodol.	Recurrences.	Lived.	
		grammes.	c.c.	minims.		days.	
Nagana	1	200	1·35	1	1	24	
	2	150	1·4	1	1	26	
	3	145	1·8	1	0	—	*Living* 108 *days.*
	4	155	2·4	$1\frac{1}{3}$	1	26	
Surra ...	5	275	1·8	$1\frac{1}{3}$	0	—	*Living* 122 *days.*
	6	150	1·8	1	0	35	Did not die of disease.
	7	315	5·7	$1\frac{2}{3}$	4	59	
	8	150	6·8	$1\frac{2}{3}$	7	81	After second recurrence with mercury sozoiodol, succinimide of mercury was substituted, of which 0·75 mgm. was given in all.
	9	150	2·7	$\frac{2}{3}$	1	33	After first recurrence, this rat was given succinimide of mercury, of which it had 1 mgm. in all.

Table IV gives the results of treatment with atoxyl and lactate of mercury, which are less good than those with succinimide and soziodolate of mercury. This drug has the disadvantage of being very irritating to the tissues, and also of forming a precipitate when mixed with atoxyl. The solution used was the ordinary hypodermic solution (2·5 per cent.) prepared by Martindale.

Table IV.—Nagana and Surra Rats treated with Atoxyl and Lactate of Mercury. Average duration of untreated diseases, 5·5 and 6·9 days respectively.

Disease.	No.	Weight.	Quantity of 5 p. c. atoxyl.	Quantity of lactate of mercury.	Recurrences.	Lived.	
		grammes.	c.c.	minims.		days.	
Nagana	1	175	1·85	1	1	25	
	2	150	2·1	1	1	26	
	3	150	1·5	1	0	23	
	4	175	3·65	1	1	31	This rat had 0·5 mgm. of succinimide of mercury after first recurrence.
Surra	5	200	3·9	1	1	32	
	6	275	3·45	1	1	30	
	7	175	1·2	1	0	21	Did not die of disease.
	8	315	3·6	1	1	24	
	9	125	3·0	$\frac{2}{3}$	2	35	This rat had 1 mgm. of succinimide of mercury after first recurrence.

Table V gives the results of a few experiments made with atoxyl and Donovan's solution (liq. arsenic et hydrarg. iodid.) which are somewhat encouraging. During the last month a fresh and more extensive series of experiments have been started. The poisonous effects of Donovan's solution show themselves in the intestines, which become intensely inflamed, whereas the effects of too large doses of succinimide of mercury show themselves principally in the kidney, in the form of an acute inflammation going on to necroses with multiple hæmorrhages.

Table V.—Nagana and Surra Rats treated with Atoxyl and Donovan's Solution. Average duration of untreated diseases, 5·5 and 6·9 days respectively.

Disease.	No.	Weight.	Quantity of 5 p. c. atoxyl.	Quantity of Donovan's solution.	Recurrences.	Lived.	
		grammes.	c.c.	minims.		days.	
Nagana	1	130	1·8	5	2	38	This rat had 2·5 c.c. 1/400 monophenylarsenic acid after first recurrence; this removed the trypanosomes from the blood, but a second recurrence took place 11 days later.
Surra	2	175	3·7	17	4	—	*Living 67 days.*
	3	150	2·4	15	3	72	
	4	200	2·25	4 +0·5 c.c.	1	28	The 0·5 c.c. of Donovan's solution was given to test dosage.* Next day the rat became very ill, and died two days after with acute enteritis. No kidney lesion.
	5	190	2·25	5	1	23	
	6	300	1·0	8	0	—	*Living 36 days.*

* See paper by Drs. Moore, Nierenstein, and Todd, before referred to.

Table VI gives the results of a series of experiments made with atoxyl and iodipin. This latter is a solution, or mixture, of iodine in oil of sesame, made by Merck, who kindly placed some at our disposal, and it is stated to be unirritating, and it can be given in large doses with safety. The results are sufficiently encouraging to suggest a further trial of this combination. The oil takes a very long time to get absorbed; it becomes colourless from absorption of the iodine, and appears to cause no irritation in the tissues.

Table VI.—Nagana and Surra Rats treated with Atoxyl and Iodipin. Average duration of untreated disease, 5·5 and 6·9 days respectively.

Disease.	No.	Weight.	Quantity of 5 p. c. atoxyl.	Quantity of iodipin.	Recurrences.	Lived.	
		grammes.	c.c.	c.c.		days.	
Nagana	1	275	4·9	7·5	2	35	
	2	150	5·4	9·5	5	71	
	3	150	1·8	6·0	0	26	
	4	195	1·5	2·0	0	112	Did not die of disease.
	5	175	2·1	6·0	2	23	Did not die of disease.
	6	275	4·0	7·5	0	29	Did not die of disease.
	7	175	4·5	7·0	3	44	
Surra...	8	175	1·9	4·0	0	57	Had eight young towards end of treatment.
	9	195	1·5	2·0	0	—	*Living* 132 *days.*
	10	195	1·5	2·0	0	—	*Living* 132 *days.*
	11	143	6·6	12·0	2	44	
	12	250	4·1	4·0	1	29	Did not die of disease.
	13	250	4·0	4·0	2	24	
	14	215	4·5	6·5	0	—	*Living* 103 *days.*
	15	165	2·9	4·5	2	33	
	16	150	3·3	6·0	3	31	Did not die of disease.
	17	150	1·8	2·5	1	20	Did not die of disease.

The Production of an Atoxyl-proof Race of Trypanosomes in Rats.

This phenomenon is of great biological and therapeutical interest, and needs much further research yet, both for its explanation, and its bearing on treatment.

In certain rats, after treatment with atoxyl, a number of recurrences may take place—we have seen as many as seven; these recurrences at first yield to treatment with atoxyl, but as the case progresses towards its end, the recurrences occur at shorter and shorter intervals, until one comes to a time when they can be no longer affected by atoxyl. The trypanosomes in this case have become atoxyl-proof. Ehrlich succeeded in producing an atoxyl-proof strain of Nagana in mice, and we have succeeded in producing atoxyl-proof strains of both Nagana and Surra in rats. One point of importance biologically is that these atoxyl-proof trypanosomes do not lose their resistance to atoxyl on transference into other rats, and we have carried this strain on in Nagana through a series of eight successive rats, and in Surra through a series of six rats without any loss of their atoxyl-proof properties. When treated with atoxyl, even in large doses, multiplication goes on just as rapidly as in untreated animals, and the rats die at the usual time. Our cases have been given atoxyl to test them, and have been then treated with various substances, as set forth in the table below. Of these substances, trypanroth and some form of mercury have given the best results. In thinking of human trypanosomiasis in this connection, the danger of the production of an atoxyl-proof strain will be at once apparent: so that atoxyl should be given to human beings with the greatest care and forethought.

The following Table VII summarises the few results we have obtained so far. Rat No. 1 was inoculated from a Nagana rat which had become uninfluenced by atoxyl after the third recurrence, before which large and repeated doses had been given: and Rat No. 11 was inoculated from a Surra rat in a similar condition.

Table VII.—Summary of Results obtained with Atoxyl-proof Strains of Nagana and Surra in Rats.

Disease.	No.	Weight.	Trypanroth 1 per cent.	Succinimide of mercury.	Donovan's solution.	Dichloro-benzidine + acid H 1 per cent.	Paratolyl-arsenic acid 1/400.	Lived.	
		grammes.	c.c.	milligrammes.	minims.	c.c.	c.c.	days.	
Nagana	1	130	—	—	—	—	—	6	Treated only with atoxyl.
	2	150	—	—	—	—	—	6	Treated only with atoxyl.
	3	140	—	0·25	—	—	—	6	Did not influence the course of the disease.
	4	250	5	0·5	—	—	2	17	Trypanroth caused a disappearance of the trypanosomes for 6 days: paratolylarsenic acid did not affect them.
	5	200	6	0·5	—	—	2	11	Neither trypanroth nor paratolylarsenic acid did more than restrain for a few days the multiplication of the trypanosomes in this case.
	6	175	—	0·75	—	6	1	10	Dichloro-benzidine + acid H caused the trypanosomes to disappear for 3 days.
	7	150	—	—	—	—	2	6	Trypanosomes were not affected.
	8	125	4	—	8	—	—	14	Trypanosomes driven out of the blood for 5 days.
	9	175	6	—	6	—	—	12	Trypanosomes driven out of the blood for 3 days.
	10	225	3	—	4	—	—	10	Trypanosomes only disappeared the day before death.
	11	125	—	—	—	—	—	3	Did not die of disease.
Surra ...	12	150	3	—	—	—	—	5	
	13	125	3	—	—	—	—	6	
	14	105	3	0·62	—	—	—	10	

The Pressure of Bile Secretion and the Mechanism of Bile Absorption in Obstruction of the Bile Duct.

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In the course of a series of experiments which were made for the purpose of determining the mode of entry of bile into the lymphatics of the liver during obstruction of the common bile duct we had occasion to measure the pressure of bile secretion in several animals. Our results were so different from the figures generally given that we have ascertained the maximum pressure of bile secretion in a number of dogs, cats, and rabbits, in two guinea-pigs, and in two monkeys. The first part of the present paper deals with the pressure of bile secretion; in the second part the mechanism and site of bile absorption are discussed.

We are indebted to Professor Schäfer for help and advice in our work. The expenses of the research have been defrayed by a grant from the Moray Fund for the prosecution of research in the University of Edinburgh.

Part I.—The Pressure of Bile Secretion.

Previous Observations.

Estimations of bile-pressure were first recorded by Friedländer and Barisch (5). These observers found that the maximum pressure attained by the bile in guinea-pigs varies between 184 and 212 mm., measured in terms of the height of a column of bile and water. Their method of procedure consisted in ligaturing the common bile duct near the duodenum, and tying a cannula into the fundus of the gall bladder which was then brought forward into the abdominal wall. A vertical glass tube was connected to the cannula by rubber tubing, the animal placed on its side and a millimetre scale affixed to the vertical tube so that the zero mark was opposite the fundus of the gall bladder. The bile mounts in the tube at first rapidly, then with decreasing speed until a maximum is reached. The column of fluid moves up and down with the respirations of the animal, and sinks again after a maximum is attained.

Heidenhain (8) in 1868 published the results of a number of experiments on bile secretion, and among them were several in which the bile-pressure was recorded. In a curarised dog the maximum pressure attained was 146 mm. bile. In a rabbit a pressure of from 163 to 168 mm. bile was recorded, in another rabbit 115 mm. bile was the maximum, while in a third rabbit a maximum pressure of 242 mm. bile was reached.

In Hermann's 'Handbuch der Physiologie,' Heidenhain (9) gives the secretion pressure of bile in five dogs as varying from 110 to 220 mm. of a solution of sodium carbonate. A simultaneous measurement of blood-pressure in a branch of the superior mesenteric vein in each of these animals gave figures varying from 50 to 90 mm. of a solution of sodium carbonate. From these figures and those of Friedländer and Barisch, Heidenhain concluded that secretion of bile is not a mechanical effect of blood-pressure in the liver, but a vital act, and that the pressure of secretion is produced by the activity of the liver cells. Heidenhain showed, moreover, that the maximum pressure reached in any case is not an absolute measurement of the force that the liver cells can exert in secretion, but is rather the pressure at which bile secretion is balanced exactly by bile absorption.

Most text-books of physiology give Heidenhain's figures. Afanassiew (1) is stated to have recorded the maximum pressure in dogs as 275 mm. bile.

Richet (15) gives the maximum pressure in cats as varying between 158 and 264 mm. bile. These figures appear to be taken from the results of experiments by Kowalewsky (12), who finds the pressure varies from 12 to 20 mm. Hg. in curarised cats.

In 1905, Freese (3) measured the force of contraction of the gall bladder in dogs. He introduced a cannula into the gall bladder through the common bile duct and cystic duct, then tied a ligature round the common duct near the duodenum. Stimulation of the splanchnic nerve caused contraction of the gall bladder sufficient to support a column of Ringer's solution 214·5 to 313 mm. high. The method does not appear to have excluded the secretion pressure of bile. Ligature of the common bile duct near the duodenum must have brought about a rapid rise of pressure in the bile passages which would be transmitted to the interior of the gall bladder. It is possible that some of the pressure changes in Freese's experiments, ascribed by him to the musculature of the gall bladder, may have been due to changes in the rate of secretion and absorption of bile in the liver.

Methods employed in the Present Research.

The animals were anæsthetised with chloroform. Tracheotomy was then performed, and the anæsthetic subsequently continued by a tracheal tube. In some cases ether was employed after the chloroform. In all experiments the animals were kept deeply under the anæsthetic and killed by an overdose, or by opening the inferior vena cava in the thorax. After anæsthesia was complete, the animal was partially immersed in the supine position in a large bath of 0·9 per cent. salt solution kept at a constant temperature of 37° C. The abdomen was opened and the cystic duct clamped by a small strong clip near the gall bladder. A silver cannula was inserted into the common bile duct and tied in position there. A fine piece of rubber tubing was slipped over the end of the cannula and attached to the lower end of a vertical piece of glass tubing 1 mm. in internal diameter. A millimetre scale was attached behind it, and so arranged that it and the glass tube could be raised or lowered to adjust the zero mark. The rubber tubing had in it a **T**-piece, from the third limb of which another piece of tubing led off to the side of the bath, and was so arranged that when open the drops of bile fell on an electric marker, by means of which the rate of secretion at zero pressure could be recorded on a slowly moving drum. The abdomen was freely opened, so that movements of the diaphragm affected the pressure as little as possible; in this way the respiratory spring of the column of bile was diminished and rarely exceeded 1 or 2 mm. The opening in the abdomen was covered with a sheet of cotton wool, and the animal was then entirely immersed, with the exception of the head and neck, in the warm salt solution. The vertical glass tube was adjusted so that the zero mark was at the level of the hepatic duct at the portal fissure. Part of the liver was thus above the level of the zero mark, but the greater part of it was at a lower level. This we believe to be as exact a method as that adopted by Friedländer and Barisch. It is certainly advantageous to maintain the temperature of the animal by warm saline when the abdomen is open, seeing that the experiment may be of some considerable duration.

In some animals we also determined the blood pressure in the splenic vein as near the portal vein as possible. The pressure was recorded by means of an apparatus similar to that described for recording the bile pressure. In this case a **T**-tube led to a pressure bottle containing a saturated solution of sodium carbonate, and the apparatus was filled and the pressure recorded in terms of a column of that solution. Before removing the clip from the vein after insertion of the cannula, the fluid was allowed to fill the vertical tube to a height approximating to the probable blood pressure; the pressure bottle was then shut off by a clip, and the clip on the splenic vein removed. The method was found to give good results.

Occasionally we made intravenous injections of extract of duodenal mucosa or of bile into a jugular vein.

In beginning an observation we usually recorded the rate of flow of bile secretion at zero pressure by the marking of drops in the way described on a slowly moving drum. After a time the tube leading to the dropper was clamped, the bile began to rise in the vertical tube and the height reached was recorded every five minutes, and in some cases every minute. Readings were continued for some time after a maximum pressure had been registered. We frequently allowed the bile to flow again at zero pressure, and after a time recorded the rate of secretion again. In some animals several estimations of the maximum pressure were made with intervals between of secretion at zero pressure. In a few cases we took simultaneous records of bile and pancreatic pressures; the latter we intend to deal with in a subsequent paper.

Results Obtained.

The bile pressure was measured in 19 cats. One of these was unhealthy and suffering from diarrhœa. The maximum bile pressure in this cat was 210 mm. bile, and was the lowest of the series. The lowest maximum of the other 18 was 240 mm., and the highest pressure of any we recorded was 373 mm. The average maximum pressure for 19 cats was 304·4 mm. bile. The subjoined table gives the figures for each animal.

Cat.	Sex.	Weight.	Maximum pressure of bile.
		grammes.	mm.
A	Male	3300	366
C	"	2850	330
D	Male (castrated)	2900	280
E	Female	2650	373
F	Male	2600	310
G	"	2950	335
H	Female	2500	321
I	Male	3200	336
J	"	2750	282
L	Female	2430	289
M	Male	3100	210
N	Male (castrated)	3720	310
O	Female	2500	325
P	Male	3200	315
Q	"	2850	319
R	"	3850	240
S	Female	2600	272
T	"	2900	244
U	"	2300	327

Measurements of bile pressure were made in eight dogs. The lowest maximum recorded was 243 mm. bile; the highest was 342 mm., figures considerably in excess of those given by Heidenhain. The lowness of the readings obtained by Heidenhain in the dog may possibly be due to his having employed curari. The average pressure in the eight dogs we experimented on was 300 mm. bile. The table presents the figures for each animal.

Dog.	Sex.	Weight.	Maximum pressure of bile.
		kilos.	mm.
A	Male	10·5	328
B	”	13·0	295
C	”	12·6	243
D	Female	11·5	285
E	Male	12·2	342
F	”	11·0	285
G	”	9·25	304
H	”	10·5	315

Four ordinary rabbits gave us maximum bile pressures ranging from 181 to 204 mm. bile. The average pressure was 197 mm.

In two Belgian hares pressures of 225 mm. and 306 mm. bile respectively were recorded. The latter animal was a large one, and the rate of bile secretion was rapid.

The subjoined table gives the details.

Animal.	Sex.	Weight.	Maximum pressure of bile.
		grammes.	mm.
Belgian hare A	Female	2800	225
Ordinary rabbit B	Male	2100	203
Ordinary rabbit C	Female	1900	181
Belgian hare D	Male	3300	306
Ordinary rabbit E	Female	2700	200
Ordinary rabbit F	”	2250	204

We made two estimations in guinea-pigs, but found these animals difficult to work with. A male guinea-pig, weighing 480 grammes, gave us a maximum bile pressure of 122 mm.; a female, weighing 650 grammes, gave a maximum of 154 mm. bile. These figures are less than those given by Friedländer and Barisch. Both guinea-pigs took the anæsthetic badly, and died before many observations had been made. Our figures are for this reason in all probability lower than the average.

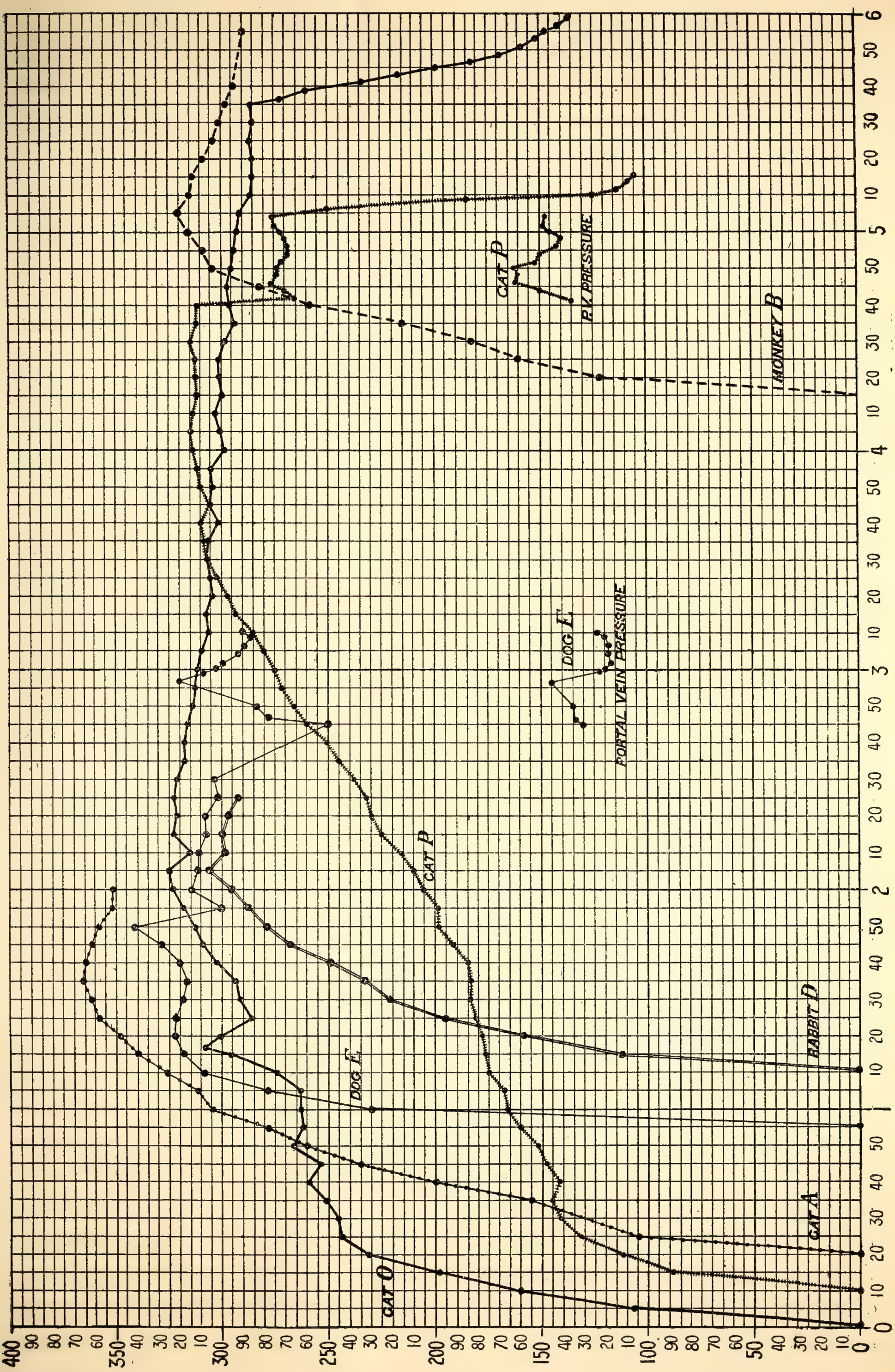
In one monkey (*Macacus rhesus*) the maximum bile pressure was found not to exceed 220 mm. bile. This animal was unhealthy, and had suffered for some time from diarrhœa. In another monkey (*Macacus rhesus*) the bile pressure rose to 321 mm. bile.

The highest bile pressures were recorded in cats, 373 mm. bile in Cat D being the greatest. The average maximum for cats, 304·4 mm., in our series corresponds closely with the average maximum for dogs, 300 mm. Rabbits have a lower pressure, in round numbers about 200 mm. bile. In a healthy monkey the maximum pressure was 321 mm. Bile pressure in the monkey probably averages about the same as that in the cat and dog.

In all our experiments the pressures recorded are measurements of the pressure in the common bile duct in terms of a vertical column of bile. The gall bladder was occluded in every case, so that its contractions could have no influence on the pressure; that the occlusion was complete was verified in every animal after death. The free opening of the abdomen under warm normal saline prevented extraneous pressure on the liver by abdominal and diaphragmatic movements; consequently, little oscillation of the column of bile ever occurred, and the readings may be accepted as indicating a true secretion pressure.

Graphic representations of the rise of bile pressure and its time relations are interesting. The accompanying chart gives several examples of pressure curves. In it the figures on the left-hand side denote the pressure in terms of millimetres of bile, while the figures below the chart represent time in five-minute intervals.

As a general rule, the bile pressure rises rapidly at first, the rate depending chiefly on the rate of bile secretion. After reaching a pressure of 100 mm. or thereabouts, the rise of pressure begins to slow down. Cat A, Dog E, and Rabbit D (see chart) gave typical pressure curves. In these animals the bile pressure rose rapidly, but the amount of rise diminished at every reading until a maximum was reached, after which the pressure began to fall. The curve of pressure in Cat P represents another type in which, after attaining a certain height, the pressure remains stationary or even falls, but only temporarily, afterwards rising gradually, but slowly. In this cat the maximum pressure, 315 mm., was attained 3 hours 55 minutes after commencement of the experiment. We had several examples of this slow rise to a maximum. In the chart is also placed a short record of the height of portal vein pressure of Cat P; the large drop in bile pressure was due to interference with the portal circulation in putting the cannula into the splenic vein; some of the sodium carbonate solution also entered the circulation. A short record of portal vein pressure in Dog E is also given. A simultaneous reading of bile pressure and portal vein pressure shows that the two coincide in their variations. Rise of blood pressure is accompanied by a rise of bile pressure; fall of blood pressure by a fall of bile pressure. Changes in blood pressure in the portal vein at once show themselves in the rise or fall of bile pressure. The rapid


CAT C
CAT A
CAT D
DOG E
RABBIT D
MONKEY B
CAT D
P.V. PRESSURE
DOG E
PORTAL VEIN PRESSURE
400 90 80 70 60 350 40 30 20 10 300 90 80 70 60 250 40 30 20 10 200 90 80 70 60 150 40 30 20 10 100 90 80 70 60 50 40 30 20 10 0
0 10 20 30 40 50 1 10 20 30 40 50 2 10 20 30 40 50 3 10 20 30 40 50 4 10 20 30 40 50 5 10 20 30 40 50 6

variations thus brought about point to a mechanical explanation of their occurrence. Heidenhain argued that bile absorption took place with greater rapidity at a low blood pressure, while the rate of secretion by the liver cells was also diminished. No doubt this is a potent factor in the long run, yet the slight and rapid changes in pressure that occur must be mechanical. Stimulation of the vagus nerve, for instance, causes a rapid and simultaneous fall of both bile pressure and portal vein pressure. In Dog C, with bile pressure 204 mm., and portal vein pressure 107 mm., stimulation of left vagus by faradic current for five seconds caused an immediate fall of bile pressure to 195 mm. and blood pressure to 95 mm. One minute later bile pressure had risen to 214 mm. and blood pressure to 115 mm.

Injection of extract of duodenal mucous membrane into a vein frequently caused a rise of bile pressure, and was found to increase the rate of secretion at zero pressure. It was not always successful, and often, while found to be very active on pancreatic secretion, had no effect on bile secretion or pressure.

The injection of bile into a vein was invariably attended by increased rate of secretion. It usually brings about an increase of bile pressure; but if the latter is high at the time of injection very little effect is noticed.

The animals we experimented on were, with a few exceptions, well nourished, and had been fed in the morning some hours before being anæsthetised. The rate of bile flow as determined before estimation of bile pressure was found to be very variable. A rapid rate of secretion is not essential to the production of a high pressure, but it influences the form of the pressure curve. The size and weight of the animal does not appear to influence bile pressure, provided the animals are of the same species.

We did not take many subsequent records of bile pressure in the same animal. The rate of secretion of bile is usually diminished after the bile duct has been obstructed for some time, but not invariably; we have obtained records of bile flow in which the rate has been increased. Subsequent records of bile pressure give as a rule lower readings than the first, as was pointed out by Friedländer and Barisch. An exception may be quoted. In dog D a maximum pressure of bile of 285 mm. was recorded. After release of the bile from the bile duct a record of bile flow was taken, and, half an hour later, secretion was more rapid than it had been prior to the first experiment. A second record of bile pressure, taken an hour after the first, reached a maximum of 305 mm. bile, an increase of 20 mm. pressure. A lower second reading is not therefore essential, and with an increased rate of secretion by the liver cells there may be, as in this case, a higher pressure reached.

Part II.—ABSORPTION OF BILE FROM THE BILE PASSAGES IN THE LIVER.—PREVIOUS OBSERVATIONS.

Ligature of the common bile duct in the living animal is followed sooner or later by the presence of the constituents of bile in the blood. It was at one time believed that the bile enters the blood directly in the liver. Haller (6) stated that communications exist in the liver between the hepatic veins and bile ducts, and in support of this adduced the fact that injection of the bile ducts with fluid is followed by its escape into the veins. Saunders (16), in 1795, published the results of experiments performed on dogs. In a living dog he ligatured the common bile duct and killed the animal two hours afterwards. He found the lymphatics of the liver distended with bilious fluid and the thoracic duct filled with moderately diluted bile. There was no appearance of jaundice as indicated by the conjunctiva, and he stated that it was probable that bile was only just beginning to enter the blood vessels.

Tiedemann and Gmelin (17), in 1827, recognised that most of the observations of Saunders were correct. They found jaundice appeared not earlier than the third day after ligature. Frerichs (4) noted a yellow tinge of the conjunctiva first appearing between the 60th and 70th hour after ligature. He challenged the statement of Saunders that bile is found in the blood after two hours.

That the bile is absorbed by the lymphatics of the liver in obstructive jaundice has received abundant confirmation from the works of V. Fleischl (2), Heidenhain, Vaughan Harley (7), and many other observers. Heidenhain determined experimentally the site of the escape of bile from the bile channels into the lymphatics. He injected a solution of indigo-sulphate of soda into the bile ducts and found that the skin, mucous membranes, and urine were rapidly coloured blue. Examination of sections of the liver showed him that there was no coloration of the interior of the lobules, the blue was present in the interlobular bile ducts, but not in the bile capillaries. Secretion of bile must have been going on while absorption was taking place. Heidenhain concluded that the bile escapes from the interlobular bile ducts into connective-tissue spaces and is carried away by the lymphatics.

Most observers, and especially Vaughan Harley, have insisted that in obstructive jaundice the bile reaches the blood solely by the lymphatics. Nauwerck (14), on the other hand, denies this and believes that the bile enters the blood directly through fine channels in the liver cells.

Mall (13) has recently stated that when the lymphatics of the liver are injected from the bile duct there is always an extravasation in the centre of the "portal unit." Extravasation does not take place from the bile capillaries, but only from the duct as it communicates with the capillaries, as well as from the larger bile ducts. Mall finds that the lymphatics of the liver do not drain all portions of the liver, but only those parts formed by the centres of the "portal units." He states that there are no lymphatics in the centres of the lobules, nor at the nodal points.

In a paper (11) published last summer we gave reasons for concluding that there are no lymphatics in the liver lobules at all ; they are confined to the portal spaces, *i.e.*, to the centres of the "portal units" of Mall.

The question of bile absorption resolves itself into that of the mode of formation of lymph in the liver. If there are no lymphatics in the lobules of the liver, and the production of liver lymph is to be regarded as purely mechanical, its source must be confined to the portal spaces. In this case the interlobular veins must be extraordinarily permeable to allow the passage outwards into the lymphatics of so large an amount of concentrated lymph. On the other hand, if the liver cells take part in its formation, the lymph must be secreted at the periphery of the lobules, and if all the cells in the lobule participate there must be something of the nature of a communication from cell to cell throughout the lobule. Given such a mechanism, the secretion of lymph may be regarded as a vital act on the part of the liver cells. We hoped that we might by our experiments throw some light upon this question.

Methods Employed.

After determining in the manner already indicated the maximum pressure of bile secretion in an animal we allowed the obstructed bile to escape from the bile passages. A piece of rubber tubing leading from a reservoir containing carmine gelatine was then slipped over the cannula in the common bile duct, and the bile passages were injected with carmine gelatine at pressures varying in different animals. We made use of an apparatus by means of which we could inject at pressures measured in terms of the height in millimetres of a vertical column of water. The difference in pressure between similar heights of a column of bile and a column of water being practically negligible, the pressures may be regarded as sufficiently alike. In most cases we employed a pressure of injection not exceeding the maximum of bile pressure previously recorded in the animal. In some cases the injection was continued and the bile duct ligatured before the animal was killed. In others the injection was made *post mortem*, the animal being killed by an overdose of chloroform, or by opening the inferior vena cava above the diaphragm so as to avoid a rise of blood pressure in the liver. Sometimes we used an injection pressure higher than the maximum bile pressure, in other cases a lower pressure was employed. Injection was continued for periods of time varying from one to six hours.

On completion of the experiment, the bile duct was ligatured and the liver removed and placed in cold 10-per-cent. formalin. After a time it was cut up, fixation completed in formalin, and pieces cut in paraffin. Sections were lightly stained with hæmatoxylin and examined.

Results Obtained.

Injection of the bile duct at the maximum pressure of bile secretion was in every case sufficient to fill the larger bile ducts with carmine gelatine. Where the animal was living throughout the experiment the secretion of bile continued, the lymph vessels in the portal fissure were distended with bile-stained lymph, and the yellow colour could in some cases be seen extending into the thoracic duct. In several experiments both in the cat and dog carmine gelatine was found in the lymph trunks of the liver and in the portal lymph glands. Whenever injection mass appeared in the lymph trunks outside the liver the injection had been at a comparatively high pressure or had been continued for some time. In Cat A, the maximum bile pressure of which was 366 mm., the injection pressure was 410 mm. for 40 minutes, and was then raised to 520 mm. water for 30 minutes longer. The animal was living throughout the time of injection, and carmine gelatine was freely mixed with lymph both in the portal lymphatics and thoracic duct. In Dog A, maximum bile pressure 328 mm., the injection was at a pressure of 400 mm. water for three hours (dog living). In this case also carmine gelatine was present in portal lymphatics and thoracic duct. Cat L, maximum bile pressure 289 mm., was injected for six hours at a pressure of 120 mm. water. The animal was dead before injection began, but the inferior vena cava had been ligatured above the diaphragm; carmine gelatine was found in the portal lymphatics on completion of the experiment.

We attempted to ascertain the time at which bile appears in the portal lymph trunks after ligature of the bile duct. For this purpose we clamped the cystic and common bile ducts in a cat, opened a lymph trunk near the portal fissure and gathered the lymph every two minutes by pieces of blotting paper which had previously been soaked in a solution of cane sugar and dried. After drying the lymph-filled pieces we tested them with strong sulphuric acid for bile acids. We were not satisfied with the reaction, but the lymph trunks issuing from the liver were strongly bile-stained one hour after obstruction of the bile duct. Heidenhain found indigo-sulphate of soda to appear in the lymph very soon after its injection into the bile ducts, but in such an experiment there is no guarantee that the entry has not been made by rupture of the interlobular ducts. Heidenhain indeed explained its occurrence on this supposition. It is difficult to show the presence of small quantities of bile in the blood, but in many of our experiments the urine contained bile acids. Dr. Cramer kindly tested the urine for us; Gmelin's test for bile pigment gave negative results, but bile acids were readily demonstrable. Bile acids were found in the urine two hours after the beginning of a record of bile pressure.

In animals which were kept alive during injection of the bile ducts at a pressure lower than or not exceeding the maximum bile pressure attained, sections of the liver show little injection. The larger bile ducts are filled with carmine gelatine and the injection passes into the smaller interlobular ducts. In the dog and cat very few of the bile capillaries are injected, a few are filled at the periphery of the lobules, but the injection does not extend more than one or two cells inwards. The adjoining liver cells are frequently filled with a lighter coloured (diluted) carmine gelatine. These cells appear to have been injected from the bile capillaries. The rabbit's liver shows the same appearance, but more of the bile capillaries are injected. Hering (10) noted the filling of liver cells from the bile duct. He stated that Berlin blue, when injected into the bile duct under so small a pressure that no extravasation takes place, fills only the bile capillary network in the periphery of the lobule. Higher pressure produces extravasation first into the liver cells in the form of small round drops, later irregular large drops, lastly, the whole liver cell appears blue. Further injection bursts the cell wall, and the injection flows into masses or escapes into the blood capillaries. We have noticed a similar appearance in sections of the livers examined. Injection of the bile ducts at a pressure near to or exceeding slightly the maximum bile pressure almost invariably causes extravasation into the blood vessels, and this is especially liable to occur when the injection is carried out in the dead animal. The extravasation appears to take place in the manner described by Hering by a filling of the cells at the periphery of the lobule and escape of the injection from them into the neighbouring sinusoids. In livers injected after death this filling of liver cells and escape into blood vessels renders the interpretation of the appearance produced a difficult one. The injection undoubtedly gets into the blood vessels at the periphery of the lobule. In an animal injected during life the appearance is somewhat different. In Cat E, which was injected during life for 55 minutes only at a pressure of 443 mm. water (maximum bile pressure, 373 mm.), the lymph of the portal lymphatics and thoracic duct contained carmine at the end of that period. Sections show extravasation of carmine gelatine into the cells at the periphery of the lobules, but no injection of the blood vessels as in the other livers. There has been escape into the blood vessels at the periphery of some of the lobules, and the carmine gelatine has been taken up by Kupffer's cells, and appears in them in the form of small round drops. Kupffer's cells are greatly distended with drops near the periphery of the lobule, and contain carmine gelatine in decreasing amounts towards the central vein, in the neighbourhood of which they are generally free.

The most valuable injections are those in which the bile duct has been injected at comparatively low pressures so as to avoid extravasation, and yet fill the interlobular bile ducts. If the pressure is higher, rupture occurs as stated, but there is also the appearance of injection mass having passed out of the finer interlobular ducts into connective-tissue spaces. It is probable that with a high pressure of injection most of the fluid passes out in this manner and so reaches the lymphatics. Heidenhain was undoubtedly right in assigning the site of rupture to the fine interlobular ducts leading from the lobules. When a low pressure is employed there is no trace of such rupture, although the ducts are filled, yet in this case bile is escaping from the liver by the lymphatics. In the rabbit's liver, where injection penetrates along the bile capillaries further into the lobules, one often sees a number of cells in a column suffused with diluted injection mass, as though the material were passing through them in the direction of the periphery of the lobule.

The readiness with which obstruction of the bile duct is recovered from, the bile again following its natural course when the obstruction is removed, and the observations of Heidenhain that the bile secreted after removal of the obstruction contains the same relative proportions of solids to fluid, show that no permanent damage is caused by the leakage, assuming it to be a leakage. One would hardly expect so complete a recovery were there a mechanical rupture of the wall of the bile ducts. One must rather assume that there is some physiological explanation of the absorption of bile. It is possible that the walls of the interlobular ducts are capable of allowing bile to pass out without any physical damage resulting, and that such passage cannot occur when the internal pressure is reduced, thus acting as a kind of safety valve allowing escape into the lymphatics when required. Carmine gelatine, however, does not appear capable of passing out in this way when injected at pressures too low to produce extravasation, but high enough to turn the flow of bile into the lymphatics.

Escape of bile into the lymphatics, if it occurs from the liver cells directly, can only be from the cells at the peripheral part of the lobule. Inside the lobule the only escape possible is by the natural channels, the bile capillaries, or into the fine plasmatic channels which permeate the liver cells. It is remarkable that the protoplasm of the liver cell, containing as it does the plasmatic channels and the origin of the bile capillaries, should be able to sustain so great a difference of pressure without bile passing from the bile capillaries into the blood sinusoids. That the liver cells should be capable of injection in much the same manner both from the blood vessels and from the bile ducts is a fact of extreme importance. The pressure necessary in either case is a comparatively small one. In none of our experiments on the injection of intracellular canals in the liver from the blood vessels did we find any escape of injection material from the cells into the bile passages. On the other hand, injection of the bile ducts in the dead animal appears to be invariably attended with filling of a few of the cells at the periphery of the lobule and escape into the blood vessels.

The intracellular plasmatic channels are in direct communication with the blood vessels, but when injected from the latter it is often difficult to make out in every cell the mode of entrance of the injection. It is probable that the liver cell exerts a controlling influence on the amount of plasma which passes in from the blood. We found it difficult to wash the injection mass out of the channels by a subsequent perfusion through the blood vessels of normal saline.

We suggested in our previous paper that the intracellular plasmatic channels of the liver act as an intermediate system, linking the blood vessels in the lobules to the lymphatics outside, and that the lymph passes from cell to cell on its way to the periphery of the lobule. The absorption of bile by the lymphatics in obstructive jaundice seems to us to favour this view. The mechanism of bile absorption appears to be in the liver cell. When the passage of bile is obstructed, the pressure in the bile ducts and capillaries rapidly rises, secretion continues, but is diverted in the peripheral cells of the lobules into the lymph stream; when obstruction is removed, the bile again follows its normal course. The amount of pressure which the liver cell has to sustain, and which is exerted on it when the bile duct is occluded, is comparatively low, and does not materially affect its vitality. An example of this is furnished by Dog D, already instanced, in which a maximum bile pressure of 285 mm. bile was recorded in the first experiment. The obstruction was then removed, and a record of the rate of bile flow taken. The rate of secretion half an hour later was more rapid than it had been prior to the experiment. A second record of bile pressure taken an hour after the first gave a maximum pressure of 305 mm. bile. That subsequent estimations of bile pressure are not as a rule as high as the first may quite well be explained by a diminution of activity of the liver cell, due to the length of time the animal is under the anæsthetic.

The form of the curves of bile pressure shows that there is not much absorption until the pressure has reached about 100 mm. bile. The rate of rise of pressure then begins to diminish more rapidly in some cases than in others. Absorption is at first slow, but gradually increases until its rate is as rapid as the rate of secretion when the column of bile becomes stationary.

On the death of the animal, the bile pressure, if high, sinks rapidly at first, but on approaching a pressure of about 100 mm. falls much more slowly and becomes almost stationary. The same thing occurs even if death has been caused by opening the inferior vena cava above the diaphragm. Blood pressure in the liver rapidly falls to zero, but the bile pressure falls slowly. The curve of fall of bile pressure after death is given on the chart. In Cat O, the sudden fall of bile pressure near the end of the tracing is due to death from an overdose of chloroform. When the pressure had fallen to 149 mm. bile, the inferior vena cava was divided above the diaphragm; bile pressure fell very slowly, and six minutes later was 134 mm. In Cat P, death was brought about by division of the inferior vena cava, and the pressure of bile secretion, which was 276 mm. at the time, fell rapidly at first, then at a decreasing rate. Ten minutes after the vein had been opened the bile pressure was 108 mm., and falling very slowly. If the bile had been escaping from ruptured bile ducts into the lymphatics or into the blood vessels, it is improbable that this slowing of the fall of pressure would have taken place.

The absorption of bile seems to us to be bound up with the formation of lymph in the liver, and while we advance no definite proofs in favour of Asher's view, we are inclined to think that the phenomena observed in the absorption of bile can best be explained on the hypothesis that lymph is a product of the activity of the liver cells, and that in obstructive jaundice the bile secreted leaves the liver cells with the lymph, instead of by its proper channels, the bile canaliculi.

Summary.

The maximum pressure reached by the bile in obstruction of the bile duct exceeds the figures originally given by Heidenhain. In the dog, cat, and monkey the average maximum pressure is about 300 mm., measured in terms of a vertical column of bile. The highest pressure recorded was 373 mm. bile in a cat. In the ordinary rabbit the pressure reaches about 200 mm. bile, but may exceed 300 mm. in the case of the Belgian hare.

After obstruction of the common bile duct and cystic duct, the pressure in the bile passages rises rapidly at first, but slows as a maximum is reached. Subsequent records of bile pressure after temporary release of the bile may exceed the first maximum, but are usually lower. After death the bile

pressure falls rapidly until it has reached about 100 mm. bile, after which the fall is comparatively slow, even when the blood pressure in the liver is at zero.

The height of bile pressure attained is, as Heidenhain showed, the pressure at which as much bile is secreted every moment as is taken up from the bile paths by re-absorption. The obstructed bile leaves the liver by the portal lymphatics. Injection of the bile ducts with carmine gelatine at a pressure not exceeding the maximum bile pressure is also followed by the appearance of the injected material in the liver lymphatics. The examination of sections of livers thus injected reveals the presence of the injection in the liver cells at the periphery of the lobules. Appearances suggest that the bile escapes from the liver cells through the intracellular plasmatic channels described by Schäfer, and that these channels are to be regarded as an intermediate system linking the blood vessels of the liver with the lymphatics at the periphery of the lobules.

LITERATURE REFERRED TO IN THE TEXT.

1. Afanassiew. Cited from Landois and Stirling, 'Text-book of Physiology,' vol. 1, p. 387 (1886).
2. Fleischl, v. "Von der Lymphe und den Lymphgefässen der Leber," 'Bericht über d. Verh. d. Königl. Sächs. Ges. d. Wiss., math.-phys. Kl.,' vol. 26, p. 42 (1874).
3. Freese. "The Force of Contraction of the Gall-bladder and the Course of its Motor and Inhibitory Fibres," 'Johns Hopkins Hosp. Bulletin,' vol. 16, p. 235 (1905).
4. Frerichs. 'Klinik der Leberkrankheiten,' vol. 1, p. 99 (1858).
5. Friedländer and Barisch. "Zur Kenntniss der Gallenabsonderung," 'Arch. f. Anat. Physiol. u. wiss. Med.,' p. 659 (1860).
6. Haller. 'Elementa Physiologiæ,' vol. 7, p. 81 (1776).
7. Harley, Vaughan. "Leber und Galle während dauernden Verschlusses von Gallen- und Brust-Gang," 'Arch. f. Anat. u. Physiol.,' Physiol. Abt., p. 291 (1893).
8. Heidenhain. "Weitere Beobachtungen betreffend die Gallensecretion," 'Studien d. physiol. Instit. zu Breslau,' vol. 4, p. 226 (1868).
9. *Idem.* "Physiologie der Absonderungsvorgänge," 'Handbuch der Physiologie, von Hermann,' vol. 5, pp. 268—278 (1883).
10. Hering, E. "Ueber den Bau der Wirbelthierleber," 'Wiener Sitzungsber.,' vol. 54, Abt. 1 (1866).
11. Herring and Simpson. "On the Relation of the Liver Cells to the Blood-vessels and Lymphatics," 'Roy. Soc. Proc.,' B, vol. 78, p. 455 (1906).
12. Kowalewsky. "Zur Lehre über die Mechanik der Gallenbewegung," 'Pflüger's Archiv,' vol. 8, p. 597 (1874).
13. Mall. "A Study of the Structural Unit of the Liver," 'Amer. Journ. of Anat.,' vol. 5, No. 3, p. 298 (1906).
14. Nauwerck. "Leberzellen und Gelbsucht," 'Münchener Med. Wochenschr.,' Jahrg. 44, p. 29 (1897).
15. Richet. 'Dictionnaire de Physiologie,' Paris, vol. 2, p. 155 (1897).
16. Saunders, W. 'A Treatise on the Structure, Economy, and Diseases of the Liver,' pp. 88—91 (1795).
17. Tiedemann and Gmelin. 'Die Verdauung,' vol. 2, p. 40 (1827).

Further Studies of Gastrotoxic Serum. (*Progress Report.*)

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(Communicated by Professor S. Martin, F.R.S. Received June 10,—Read June 27, 1907.)

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[Plate 7.]

In two (1 and 2) previous communications to the Royal Society I was able to demonstrate the production of a gastrotoxic serum by the injection of the mucous membrane of the stomach of one animal into another. In this way, a heterogastrotoxin was formed by injecting the stomach cells of the guinea-pig into the rabbit, and an isogastrotoxin by injecting the stomach cells of the rabbit into the rabbit. I also showed that a human gastrotoxin could likewise be produced by injecting human stomach cells into another animal.

The heterogastrotoxin possesses poisonous properties for the tissues of the variety of animal whose stomach has been used for injection, but the isogastrotoxin does not. The latter, however, is toxic for the tissues of a nearly allied animal.

The gastrotoxin, on injection into an animal for which it is poisonous, produces necrotic patches in the mucous membrane of the stomach, which develop into ulcers. In the test-tube the serum also produces effects upon the tissues of the animal concerned. It was proved to be of a complex nature, and to be capable of effecting the following reactions:—

1. Hæmolysis of the red blood corpuscles.
2. Precipitation of the soluble proteids of the stomach cells, and also of other proteids of the body.
3. Agglutination of the gastric protoplasmic granules.
4. A more or less definite hyaline transformation of the intact gastric cells.

The present communication will deal with the four following points:—

1. The multiplicity of the precipitins.
2. The identity of the substances producing the precipitating and agglutinating reactions.
3. The production of immunity to the gastrotoxin.
4. The *rôle* which the gastric juice plays in the actual formation of the necrotic patches and of the subsequent gastric ulceration.

The gastrotoxic serum was in all cases obtained by injecting the washed stomach cells of the guinea-pig into the rabbit, the blood serum of the rabbit developing toxic properties for the guinea-pig's tissues.

1. *The Multiplicity of the Precipitins.*

My object was to ascertain whether the same precipitin or different precipitins were concerned in the precipitation of the various proteids.

Method.—The proteid solutions examined were albuminous extracts of the gastric, hepatic, and intestinal cells, and the blood serum of the guinea-pig. The extracts were made by grinding up the cells to a pulp, shaking up the latter with salt solution, and filtering through a Berkefeld filter. The proteid solutions were all made of such a strength that a faint cloud was formed on testing them with potassium ferrocyanide and acetic acid. It is necessary to use the solutions of the same strengths, because the precipitate is soluble in excess of the precipitable substance, and therefore if albuminous fluids of different strengths are employed, erroneous results will follow.

If equal volumes of such a proteid solution and anti-serum be mixed together and incubated for four hours, and if the precipitate which forms in the solution be removed by centrifugalisation, the resulting solution, which will consist of a two-fold dilution of anti-serum, will be found to be incapable of precipitating any further the proteid solution. In this way one can easily remove the precipitin for any particular proteid, and the solution can then be tested with regard to its power of precipitating a different proteid. In all cases, of course, a control must be prepared, so as to prove that the precipitin has been actually removed.

In this way the precipitins for each of the four proteid solutions mentioned above were removed, and the resulting serum diluted to different degrees and tested as to its capacity of precipitating the three remaining proteids, with the following results:—

Removal of the Precipitin for Stomach-cell Proteid.—A solution of gastrotoxin from which the precipitin for stomach-cell proteid has been removed is thereby rendered quite incapable of precipitating the proteids of liver cells, intestine cells, or blood serum.

Removal of the Precipitin for Liver-cell Proteid.—Removal of the precipitin for liver-cell proteid slightly weakens the power of the gastrotoxin to precipitate the proteids of stomach cells and blood serum, and it completely deprives it of the power of precipitating intestine-cell proteid.

Removal of the Precipitin for Intestine-cell Proteid.—Removal of this precipitin renders the gastrotoxin incapable of precipitating liver-cell proteid, but only weakens its precipitating power for the proteids of stomach cells and blood serum.

Removal of the Precipitin for the Proteid of Blood Serum.—If the precipitin for the blood serum be removed, the gastrotoxin is still able to precipitate all the three remaining solutions, but not quite to the same degree as before.

It appears, therefore, that the precipitins of gastrotoxic serum are multiple and to a great extent specific, but that their actions overlap to some extent. The gastrotoxic precipitins for liver-cell and intestine-cell proteids appear to be more nearly allied than the others, if not identical. In confirmation of the above statements I may say that if the blood serum of an immunised rabbit be examined every day, it will be found that the first precipitins to appear in the serum are those for the proteids of the stomach cells and blood serum, and that the precipitins for the liver-cell and intestine-cell proteids appear at a later date during the process of immunisation.

These results are in agreement with the statement of Nuttall (3) and others to the effect that precipitins are to a large extent, but not absolutely, specific.

2. *The Identity of the Substances producing the Precipitating and Agglutinating Reactions.*

The precipitin for stomach-cell proteid was removed from a solution of gastrotoxin in the way described above and the capacity of this solution to agglutinate gastric-cell granules then tested. In order to prove that the precipitin was removed from the solution, a control was in each case prepared, in which stomach-cell proteid was added to the solution instead of gastric granules.

In this way I have been able to show that in the absence of the precipitin no agglutination of gastric granules occurs, in other words the gastrotoxin does not contain a specific agglutinin for the gastric granules. When unwashed granules are used for the performance of the agglutination test, the gastrotoxin precipitates the albuminous fluid in which the granules float and the latter are agglutinated by this precipitate. When washed granules are used, the gastrotoxin no doubt produces some change of a coagulative nature in the superficial layers of the granules and they are therefore agglutinated as they sink to the bottom.

On the other hand there is no doubt that the gastrotoxic agglutinin for red blood corpuscles is a specific substance, because if the gastrotoxic precipitins be removed as described above, the resulting solution still agglutinates red blood corpuscles in exactly the same strengths as untreated gastrotoxic serum does. The serum is, of course, heated to 55° for half an hour before performing this test, in order to prevent hæmolysis. My results disagree with those of observers such as Deutsch (4) and others, who hold that specific agglutinins are formed for the protoplasmic granules of cells, and who make no mention of any precipitating action. Their position, according to my experiments, is untenable.

3. *The Production of Immunity to Gastrotoxin.*

The effect of repeated injection of gastrotoxic serum is to produce immunity to the gastrotoxin.

It seemed that a determination of the results obtained from the continued administration of gastrotoxin would be interesting from two points of view:—

(1) With regard to whether chronic ulceration of the stomach could be produced in this way, or whether the animal would become immune.

(2) If the animal became immune, whether it would develop protective substances in its blood against one or all of the constituents of the gastrotoxic serum. In this way light would probably be thrown upon the actual cause of the ulceration, whether it was of hæmorrhagic origin primarily, or due to a direct action upon the gastric cells.

Method.—The method which I have found to be the most satisfactory is the intraperitoneal injection of inactive (heated to 55° C.) gastrotoxic serum.

The injected animal was, of course, the guinea-pig, and the serum was derived from rabbits immunised by injection of washed guinea-pig's stomach cells.

One injection of 5 c.c. was given each week. In order to produce a satisfactory degree of immunity, 10 or 12 injections are necessary.

In this way I have immunised 10 guinea-pigs. The immunity of these animals was tested both by experiments *in vivo* and also *in vitro.*

1. *Examination* in vivo: *Active Immunity.*—Four or five injections of the serum will not protect an animal against a lethal dose (10 c.c.) of gastrotoxin, which still produces necrotic patches in the stomach.

After an animal has, however, received 10 injections (50 c.c. serum in all) a lethal dose (10 c.c.) of gastrotoxin fails to produce necrotic patches in the stomach of that animal (fig. 1, Plate 7).

Passive Immunity.—The blood serum of an actively immune animal is capable of conferring passive immunity upon another animal.

In an experiment of this kind a lethal dose (9 c.c.) of active gastrotoxic serum was mixed *in vitro* with 6 c.c. inactive protective serum and the mixture injected into a guinea-pig weighing 360 grammes. No lesion was produced in the stomach. The control animal was inoculated with 9 c.c. active gastrotoxic serum and 6 c.c. inactive normal guinea-pig's serum. This animal showed the usual patches of necrosis in its stomach. Normal guinea-pig's serum is itself able to protect an animal to a slight extent (fig. 2).

2. *Examination* in vitro.—The examination was conducted from two points of view:—

(*a*) To find out whether the tissues of an immune animal were as susceptible to the gastrotoxin as were those of a normal animal.

(*b*) To ascertain what protective power the serum of the immune animal possessed with regard to preventing the action of gastrotoxin *in vitro.*

(*a*) *Action upon the Tissues of an Immune Animal: Blood Corpuscles.*—The gastrotoxic hæmolysin dissolves the red corpuscles of an immune animal to exactly the same degree as it does those of a normal guinea-pig.

Proteid Solutions.—The stomach proteid, intestine proteid, and even the blood serum of the immune animal are all precipitated by the gastrotoxic serum.

Stomach Cells.—The same action is seen upon the immune cells as upon the normal cells, namely, more or less clearing of the cells.

In the case of several other poisons it has been proved that the tissues of the immunised animal are still acted upon by the poison.

(*b*) *Protective Power of the Serum* in vitro.—The protective serum was, in all cases, heated to put the complement out of action.

Hæmolysin.—Hæmolysis is completely prevented by the immune serum.

The immune serum obtained from an animal after four injections prevents hæmolysis to some extent. After eight injections it has more power. After 11 injections 0·5 c.c. serum completely prevented any hæmolysis occurring in any of the tubes. (Hæmolytic strength of the serum used:—0·25 c.c. serum completely dissolved 1 c.c. 5-per-cent. suspension of guinea-pig's corpuscles.)

Probably smaller amounts than this would suffice. The experiments were only made, however, to demonstrate the presence of an anti-hæmolysin.

Precipitin.—I have not been able to show the presence of any anti-precipitin. The serum of an immune animal, on being added to the gastrotoxin in sufficient amount to dissolve any precipitate that forms, fails to prevent the precipitation of stomach-cell proteid.

Action on the Gastric Cells.—The changes in the cells are not sufficiently definite for any very accurate determinations to be made, but my impression is that the cells are as much acted upon, whether the immune serum is present or not. This point must, therefore, for the present be left an open question.

Anti-precipitins have been described, but my results fail to confirm such observations.

Anti-hæmolysins have, of course, been recognised for a long while.

4. *The Rôle which the Gastric Juice plays in the Production of Gastric Ulceration.*

I have been able to prove that the actual ulceration produced by injection of the gastrotoxic serum is brought about by the action of the gastric juice.

Although it is considered at the present day that the gastric juice plays an important part in the production of gastric ulcer, I have not been able to find any reliable experimental evidence in support of such a supposition. Of course, it is clear that if the gastric cells are killed by any means, the gastric juice will digest them, as it does any foreign proteid substance, but I refer to self-digestion of the stomach, in which the gastric juice attacks a gastric cell which shows no deviation from the normal on microscopic examination.

The whole question of self-digestion of the stomach is extremely interesting and important, but as yet very little is known about the subject.

Method.—My method is to neutralise the gastric juice with sodium bicarbonate solution before injection of the gastrotoxic serum.

In order to do this a soft rubber catheter is passed down the œsophagus of a guinea-pig into its stomach, and about 14 c.c. of a 4-per-cent. sodium bicarbonate solution introduced through a small funnel. The gastrotoxic serum is then injected into the animal's peritoneal cavity. A control pig is at the same time injected with the same amount of the same serum. I have done eight such experiments, and in all the cases in which the gastric juice was previously neutralised no necrosis of the stomach could be seen, but in all the control animals the usual black patches were visible in the mucous membrane of the stomach (fig. 3).

The stomach contents in the pigs which were previously treated with alkali were found to be strongly alkaline, whilst the contents of those not so treated were strongly acid. I am not yet prepared to state the weakest strength of the soda solution which will prevent necrosis.

I have hitherto found no microscopic change in the stomach which precedes digestion.

These experiments conclusively prove what I had previously shown in another way, namely, that the initial lesion is not a hæmorrhage, for in that case neutralisation of the gastric juice would certainly not prevent a hæmorrhage occurring. It is also well known that precipitins cause no lesion on injection, because any precipitate formed would be dissolved by the excess of the animal's fluids present. It appears to me that a functional disturbance is set up in the gastric cells which renders them susceptible to the action of the gastric juice. What that disturbance is and whether or not it bears any relationship to the slight hyaline change which I have described as occurring *in vitro,* I am not prepared to state. I think, however, that it is a different process, because the action is destroyed by heat, no visible change is to be seen in the cells, and an antibody is formed against it.

If a solution of hydrochloric acid (0·5, 1, or 2 per cent.) be introduced into the stomach previous to the injection of the gastrotoxic serum, the resulting necrotic lesions are more pronounced than they are in the control animals (fig. 4). A 2-per-cent. solution of hydrochloric acid alone produces no effect whatever when introduced into the stomach of a guinea-pig.

I think, therefore, that hyperacidity of the gastric juice must play some part in the formation of gastric ulcers; the essential factor, however, is some alteration in the cell itself, and probably this alteration may be brought about in a variety of ways.

Conclusions.

1. A gastrotoxic serum contains many precipitins which are more or less specific in their actions, but these actions overlap to some extent, and absolute specificity is unknown.

2. There is no specific agglutinin for the gastric granules, the agglutination being brought about by the precipitins.

3. By repeated injection of the gastrotoxic serum, immunity is established. The immune substance is present in the blood serum of the animal, its tissues being still susceptible to the poison.

It seems, therefore, impossible to establish a chronic lesion by gradual absorption of the gastrotoxin, if the immunising mechanism of the animal be intact. Any lesion which is produced occurs within a few hours, as I have shown. If a chronic lesion occurs as the result of the action of such a cytotoxin, the explanation is probably to be found in the perpetuation of an acutely produced lesion by some cause or other. Whether this cause be secondary bacterial infection or hyperacidity of the gastric juice remains to be proved.

4. The actual necrosis and ulceration of the stomach is produced by the gastric juice acting upon a cell which is functionally damaged. Hyperacidity of the gastric juice increases the tendency to such ulceration.

REFERENCES.

1. Bolton, 'Roy. Soc. Proc.,' vol. 74, p. 135, 1904.
2. Bolton, 'Roy. Soc. Proc.,' B, vol. 77, p. 426, 1906.
3. Nuttall, "Blood Immunity and Relationship" (text-book).
4. Deutsch, "Sur le sérum antihépatique." XIII^e Cong. Internat. de Méd., Sect. de Bact. et Parasitol., 1900, Paris, 'Compt. Rend.,' pp. 55—56.

DESCRIPTION OF PLATE.

Fig. 1.—Active Immunity to Gastrotoxic Serum.

Upper stomach: This is the stomach of the control animal, which was injected with 10 c.c. gastrotoxic serum. It shows patches of necrosis of the mucous membrane.

Lower stomach: This guinea-pig had been immunised with 10 injections of gastrotoxic serum. A lethal dose (10 c.c.) of gastrotoxic serum was injected. No lesion has resulted.

Fig. 2.—Passive Immunity.

Upper stomach: This animal received a mixture of 9 c.c. gastrotoxic serum and 6 c.c. protective serum. No lesion is to be seen.

Lower stomach: This animal received a mixture of 9 c.c. gastrotoxic serum and 6 c.c. normal serum. Necrotic patches are to be seen in the stomach.

Fig. 3.—Neutralisation of the Gastric Juice by Sodium Bicarbonate.

Upper stomach: This is the stomach of the control animal, which was injected with 10 c.c. gastrotoxic serum. It shows necrosis of the mucous membrane.

Lower stomach: 14 c.c. sodium bicarbonate solution (4 per cent.) were introduced into this stomach, and the animal was then injected with 10 c.c. gastrotoxic serum. No lesion has resulted.

Fig. 4.—Hyperacidity of the Gastric Juice.

Upper stomach: This is the stomach of the control animal, which was injected with 10 c.c. gastrotoxic serum. It shows some necrosis of the mucous membrane.

Lower stomach: 27 c.c. hydrochloric acid solution (1 per cent.) were introduced into this stomach, and the animal was then injected with 10 c.c. gastrotoxic serum. Extensive necrosis of the mucous membrane has resulted, and it is much more marked than in the control animal.

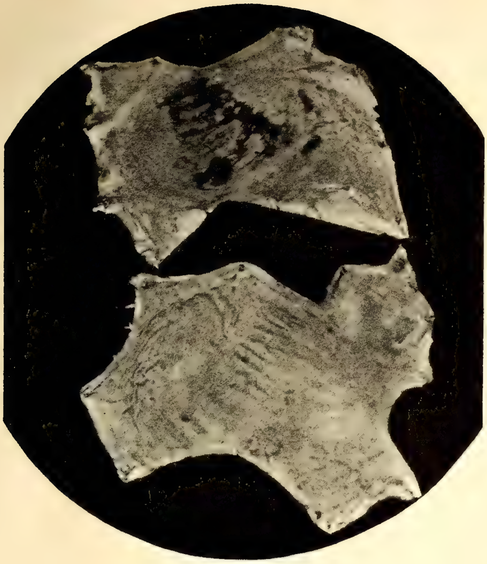

Fig. 1.

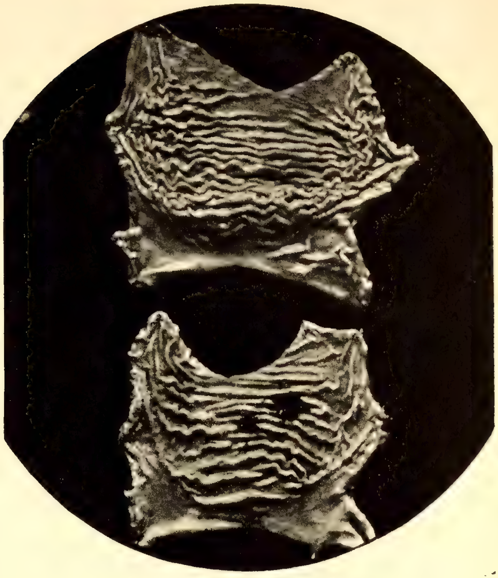

Fig. 2.

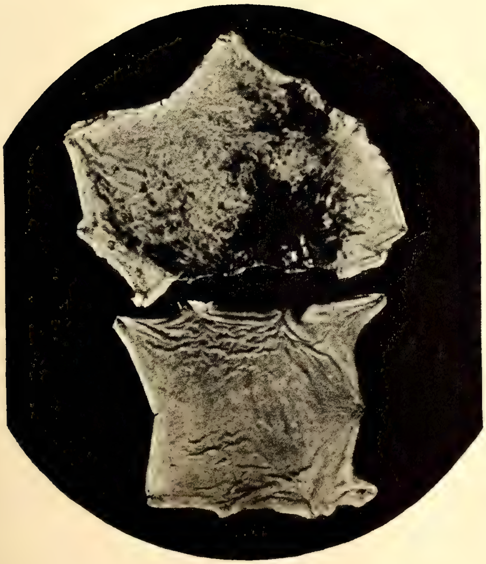

Fig. 3.

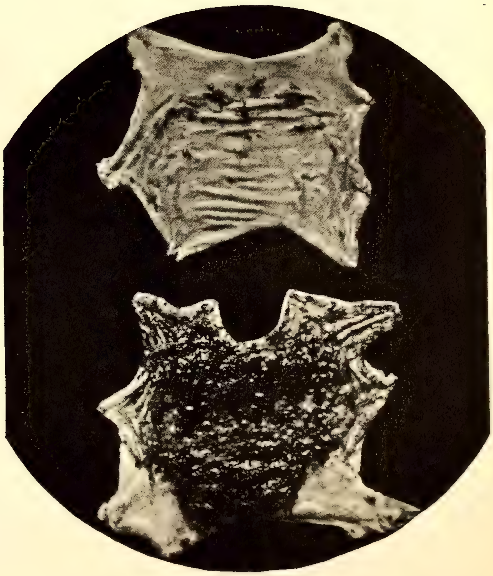

Fig. 4.

On the Relation between the Output of Uric Acid and the Rate of Heat Production in the Body.

By E. P. Cathcart, M.D., and J. B. Leathes, M.B.

(Communicated by Dr. C. J. Martin, F.R.S. Received June 22,—Read June 27, 1907.)

(From the Lister Institute of Preventive Medicine.)

The rate at which uric acid is turned out of the body is very different at different times of the day, even when the food contains no ready-made purine derivatives. It is higher during the early hours of the day than at any time, and it is considerably lower at night. The reason for this, as was pointed out by one of us,* is not likely to be that the excretory functions are depressed at night, since these functions, to judge from the total nitrogen of the urine, are more active during the first hours of sleep than at any time in the 24 hours. And since, when the diet is confined to bread, butter, and milk, the uric acid must be derived from the body substance and not from the food, it seems probable that there is some function of the body which is in abeyance during sleep and is, to a considerable degree, responsible for the output of uric acid; some function, that is to say, which is effected by chemical reactions involving the production of uric acid, and of nothing else that can be detected in the urine, unless it be phosphoric acid, and possibly in some measure creatinine.

If it is possible to identify this function, the activity of which can, on a suitable diet, be measured by the amount of uric acid excreted, it may be possible to give a clearer account of the processes by which, at the onset of fever, the temperature of the body can be sent up independently of any voluntary muscular activity; for while the temperature is rising, the output of uric acid may be four times as great as it otherwise would be.† Similarly, in the study of other pathological conditions in which uric acid plays a part, it must be of importance to be able to point to the kind of activity which is accompanied by increased uric acid production.

The following series of observations, which we have worked out conjointly, was designed to show how the reaction of the body to loss of heat on the one hand, and the heat produced by ordinary muscular exertions on the other, might affect the amount of uric acid in the urine. It was the outcome of several earlier, as yet unpublished, experiments, which pointed, though less clearly, to the same conclusions as we think may be drawn from this one.

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* J. B. Leathes, 'Journal of Physiology,' vol. 35, p. 125, 1906.

† *Loc. cit.*, p. 205.

A diet was taken by the subject of the experiment (E. P. C.) which consisted of 2 quarts of milk (2·28 L.), 12 ozs. of bread (360 grammes), 4 ozs. of cheese (120 grammes), and 2 ozs. of butter (60 grammes). This was distributed equally over six identical meals taken every three hours from 7 A.M. to 10 P.M.

The urine was collected immediately before each meal, and in each sample the total nitrogen (Kjeldahl), the creatinine (Folin), the uric acid (Folin), and the phosphoric acid (by titration with uranium acetate) was estimated, and the hourly rate of output calculated for each period of the day. The diet was taken from May 17 till June 4. On the first two days the urine was collected for 24 hours and mixed before analysis.

On the eighth day of the diet, May 24, two and three-quarter hours were spent stripped at a swimming bath. For the greater part of this time the subject of the experiment stood about, and entered the water only from time to time as the skin became dry, the object being to lose heat with as little compensatory production of heat by voluntary exertion of the muscles as possible. The rectal temperature was reduced in this way from 100°·8, at 2.50 P.M. immediately after undressing and after the 10 minutes' walk to the bath, to 97°·7 just before dressing again at 5.30. The reading at 4 P.M. was 98°·8, and at 5 P.M. 98°. The temperature of the water was 71°, and that of the air 64°. At fairly frequent intervals spasmodic attacks of shivering came on, but these were not so severe as in a "rigor." Although the body temperature fell 3° F., the greater part of this fall would have occurred even with clothing, as the observations taken every three hours throughout the whole experiment showed that a rectal temperature of 98°·8, or even slightly less, was normal for this time of day on abstaining from muscular activity. The body temperature, therefore, fell barely a degree beyond the normal range of variations, and the regulating function was not seriously overtaxed. This was the result aimed at, as a previous experiment had shown that a more severe and rapid removal of body heat introduced disturbing factors and gave a different result.

The effect on the output of uric acid was to increase it more than 80 per cent. in comparison with the mean of the four preceding days. The figures observed are given in Column C of Table I appended.

Three days later, May 27, the exposure to cold was repeated, only with this difference that active muscular exertions were introduced. The subject was stripped for two and three-quarter hours and kept his skin wet by repeated immersion in, and sponging with, cold water, T.° 59 to 60. In the intervals quick-running movements, maintained for five minutes at a time, and dumb-bell exercises, sufficient to cause pronounced hyperpnœa, were carried out. In this way actual shivering was almost entirely avoided, although the periods of immersion, longer than those on the 24th, and the sponging with water 10° colder than that at the swimming bath, the measures that is for removing body heat, were more drastic than on the former occasion. The body temperature, which was 100°·4 at 4.10 P.M. when the treatment began, was still 100° at 5.30; it was lowest (98°·8) at 6.30, but was slightly raised again (99°·1) at 6.50, when the treatment ended.

The effect on the output of uric acid, shown in Column D of Table I, was in the succeeding 24 hours to increase it, but only to about half the extent to which it was increased by the cold without muscular exertions three days before, and if the comparison be made with the output of the 24 hours immediately preceding, *i.e.*, 312 milligrammes, the increase is less than 20 per cent., and during the three hours of the treatment the output was remarkably low.

Three days later, on May 30, the subject of the experiment, dressed in winter clothing, with, in addition, a thick knitted jersey, spent the greater part of two and three-quarter hours in severe muscular exertions. At 3.10 P.M., with the rectal temperature at 99°·8, a sharp walk of 2½ miles, lasting half an hour, raised the temperature to 102°·4. Then, after an interval of a quarter of an hour for food, a heavy floor rubber was worked to and fro for half an hour, at the end of which time the temperature was 101°·8. The walking was then repeated (T.° 102·1) and finally the work with the rubber for half an hour more giving a temperature at 5.50 P.M. of 102°.

The output of uric acid fell from 311 milligrammes in the preceding 24 hours to 223 in those including and succeeding the exercise. The figures are given in Column E of Table I.

Finally, four days later, on June 4, an attempt was made to repeat the experimental conditions of the first exposure to cold on May 24 in the swimming bath. It was not possible to do this exactly, because the air temperature was 8° lower, 56° F., and at this temperature the loss of heat was sufficient to cause shivering, which was fairly continuous for two hours. The body temperature was 98°·4 at 4 P.M. as compared with 98°·8 at the same time on the earlier occasion, but at 5.30, just before dressing, it was a trifle higher than the first time, 98° instead of 97°·7. Whether these differences in the conditions account for the result shown in Column E, which is of the same character though not so pronounced as that in Column C, we are not yet prepared to say. The output in the 24 hours preceding the final exposure was 311 milligrammes, this was raised to 386 milligrammes. This is less than the output on the former occasion, but, as on that occasion, the figures for each of the periods in the 24 hours are above the average and particularly that for the period ending at 7 P.M., which included the greater part of the exposure to cold.

On May 19, the third day of the diet, the body weight was 70·5 kilogrammes; on June 4 it had risen to 73·2 kilogrammes.

The results of this experiment point to the inference that one of the factors largely concerned with the production of uric acid in the body and its appearance in the urine is the reaction of the body to loss of heat; and that a part, it may be a large part, of the endogenous uric acid is, so to speak, the chemical expression for the intensity of this reaction; further, that this reaction involves some form of activity distinct from voluntary movements of the skeletal muscles, since such voluntary movements have, on the contrary, the effect of diminishing the output of uric acid, at any rate while they are going on.

At the same time we are not prepared to say that the activity for which the chemical expression is the output of uric acid, has no other physiological expression than heat production in response to increased loss of heat. That, indeed, would not at all represent our view. For it is at any rate clear, from this experiment taken by itself, that the reaction set up by exposure to cold may last on for a considerable time after the exposure. A glance at the column in Table II, in which the output of uric acid for each 24 hours throughout the experiment is shown, makes it obvious that the interval of three days that elapsed between each abnormal phase of the experiment was not sufficient to allow a complete return of the rate of output to its value in the days before the first exposure.

The nature of the activity concerned in this reaction to cold, and certain other points arising out of the data in Table II, will be entered into more fully on another occasion.

Table I.—Showing the Rate of Output of Uric Acid, in milligrammes per hour, for the different periods of the day ending at the hours indicated in the first column.

	A. Mean of all the 16 days.	B. Mean of four normal days before exposure to cold.	C. First exposure to cold, without exercise, from 2.50 to 5.30 P.M.	D. Cold + exercise, from 4.10 to 6.50 P.M.	E. Exercise, warmly dressed, from 3.10 to 5.50 P.M.	F. Second exposure to cold, without exercise, from 2.50 to 5.30 P.M.
7 P.M.	13·1	11·5	19·5	6·2	3·6*	17·7
10 „	14·7	13·0	21·1	20·4	6·9	15·5
7 A.M.	10·9	9·2	17·1	12·4	9·2	14·7
10 „	18·6	15·3	28·7	20·7	13·8	21·6
1 P.M.	14·5	12·0	20·2	19·2	11·0	14·7
4 „	13·9	11·5	17·4	19·6	11·3	14·9
Total for 24 hrs.	321·0	259·0	475·0	369·0	223·0	386·0

* This figure is almost certainly too high.

Table II.—Total Daily Output of the various Urinary Constituents estimated.

Date, 24 hours ending 4 P.M.	Uric acid.	Total nitrogen.	Creatinine.	P_2O_5.	H_2O.	Air temperature.		Remarks.
						6 P.M.	Noon.	
	mgms.	grammes.	grammes.	grammes.	c.c.	°	°	
May 20	335	16·25	1·50	3·27	1260	49	47	
„ 21	280	17·37	1·49	3·69	1582	45	51	
„ 22	225	18·22	1·46	3·77	1544	52	53	
„ 23	259	17·40	1·51	3·74	1378	59	65	
„ 24	270	17·73	1·49	3·35	1314	67	65	
„ 25	**475**	18·81	1·44	4·04	1083	64	70	2¾ hours stripped at swimming bath at beginning of this period
„ 26	338	18·74	1·51	3·83	1295	67	57	
„ 27	312	17·57	1·48	3·64	1429	58	61	
„ 28	**369**	17·88	1·51	3·33	1295	66	57	2¾ hours' exercise stripped and cold
„ 29	314	17·59	1·49	4·20	1616	51	54	
„ 30	311	17·07	1·49	3·93	1578	52	54	
„ 31	**223**	17·98	1·57	3·65	1002	57	64	2¾ hours' exercise heavily dressed
June 1	375	18·62	1·56	4·19	984	61	64	
„ 2	354	18·95	1·54	4·06	1266	59	58	
„ 3	309	19·39	1·46	4·24	1625	54	54	
„ 4	**386**	20·11	1·43	4·12	1608	56	—	2¾ hours stripped at swimming bath
Mean of 16 days	321	18·10	1·49	3·89				

Implantation of Actively Proliferating Epithelium.

By J. O. WAKELIN BARRATT, M.D., D.Sc.

(Communicated by Professor Sherrington, F.R.S. Received July 20, 1907.)

(From the Cytological Laboratories of the University of Liverpool.)

The present investigation deals with the course of events following subcutaneous implantation of actively proliferating adult epithelium. To avoid the possibility of disturbing factors being introduced by the formation of antibodies, the epithelium was implanted into the same animal or into an animal of the same species.

The effect of implantation of normal adult epithelium has, more particularly during the last two decades, been the subject of considerable study by a large number of observers, whose investigations have shown that epithelium may be successfully implanted and an epithelial cyst result, but that the implanted epithelium shows a marked tendency to die out in its new habitat.*

In the present research, instead of using normal epithelium for implantation, rapidly proliferating epithelium was employed. It was anticipated that additional information would thereby be afforded upon two important points, namely, upon the immediate influence of implantation on the activity of the epithelial cells, and (owing to the large bulk of epithelium available for implantation) upon the influence exerted by the implanted epithelium upon non-epithelial tissue elements. Both these anticipations have been fulfilled.

The use of rapidly proliferating epithelium has been rendered possible by Fischer's discovery of the action of scharlach R upon the epithelium of the skin of the rabbit.† The extent to which epithelial proliferation occurs may be judged by a glance at fig. 2, which shows the appearance of a section of the ear after the injection of the dye, fig. 1 showing, for comparison, a normal section drawn to the same scale.‡

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* *Cp.* E. Korschelt, "Regeneration und Implantation im Tierreich," 'Verhandlungen der Gesellschaft Deutscher Naturforscher und Aerzte,' 1906, Erster Teil, S. 164.

† B. Fischer, "Die experimentelle Erzeugung atypischer Epithelwucherungen und die Entstehung bösartiger Geschwülste," 'Münch. Med. Wochenschr.,' 1906, 53 Jahrg., S. 2041.

‡ The sections represented are stained by Heidenhain's iron alum hæmatoxylin method, except in fig. 1, where methylene blue eosin is used for staining. Figs. 1 and 2 are magnified 33 diameters; the remaining figures are magnified 800 diameters.

FIG. 1.

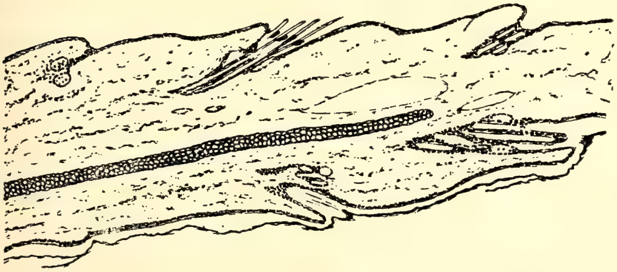

FIG. 2.

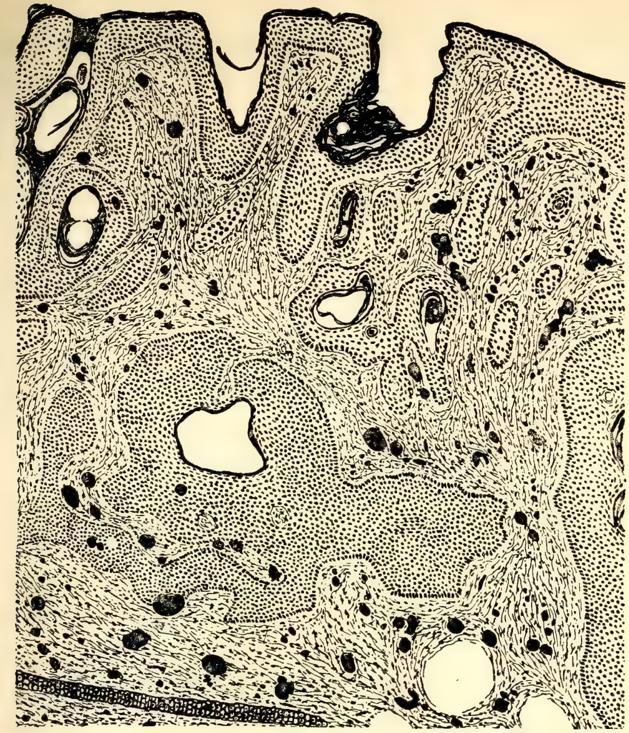

FIG. 1.—Section of normal ear of rabbit, for comparison with the succeeding figure. In the middle the cartilage is seen. Hair follicles, containing hairs, are present; also a few sebaceous glands.

FIG. 2.—Section of rabbit's ear injected with scharlach R dissolved in olive oil. The thickness of the ear has been increased about five-fold, the increase being chiefly due to irregular hypertrophy of the Malpighian layer of the surface epithelium and of the hair follicles. The stratum corneum is also hypertrophied, and considerable increase in the nuclei of the corium is present. Fat globules, stained black, are seen: some are contained in fat cells, some have come from the oil injected. The cartilage of the ear is shown in the section, and is not markedly thickened.

A series of 31 implantations of skin from the rabbit's ear, after injection of scharlach R dissolved in olive oil, was made, the site of implantation being the skin of the back (Table I). The epithelium of the skin implanted was very actively proliferating, being in the condition shown in fig. 2. The thickness of the portion of skin implanted was about 1 mm. Before removal of skin for implantation the rabbit's ear was shaved, all loose horny epidermis removed, and the surface washed with absolute alcohol, followed in some experiments by the application of a 1 in 500 solution of mercuric chloride. The latter did not, however, present any obvious advantage, nor

Table I.—Experiments in which Portions of the Skin of the Ear of the Rabbit, previously injected with Scharlach R so as to cause Epithelial Proliferation, were implanted subcutaneously.

No. of experiment.	Duration of implantation.	Condition of implanted skin at end of period of implantation.			
		Condition of epithelium.	Mitoses in epithelial cells.	Necrotic epithelium.	Scharlach R oil globules.
	days.				
1	3	In part living	Absent	Present	Present
2	6	None living	"	Abundant	"
3	6	In part living	Present	Present	"
4	6	None living	Absent	Abundant	"
5	6	"	"	"	"
6	7	In part living	Present	Present	"
7	7	"	"	"	"
8	7	None living	Absent	Abundant	"
9	7	"	"	"	"
10	7	In part living	Scanty	Present	"
11	7	"	Absent	"	"
12	7	"	"	"	"
13	8	"	Numerous	"	"
14	14	Very little living	Absent		Scanty
15	14	"	Scanty		"
16	14	"	"		Present
17	31	"	Absent		"
18	31	"	"		"
19	31	None living	"		
20	31	Very little living	"		
21	71	"	Scanty		
22	71	None living			
23	71	"			Very scanty
24	71	"			
25	80	Very little living	Scanty		
26	80	"	"		
27	80	None living			
28	80	"			
29	90	"			
30	90	"			
31	135	"			

was it in any way inimical to the success of the implantation. The portions of skin implanted were removed at the end of from three days to four and a half months. In 17 out of the 31 implantations, living epithelium was found on removal; in the remaining 14 experiments no living epithelium was present on removal.

For comparison with the above a second series of seven implantations of normal skin from the rabbit's ear (*cp.* fig. 1) was carried out (Table II). In five of these experiments living epithelium was found on examination at the end of from seven days to four and a half months.

Table II.—Control Experiments in which Healthy Living Skin of the Rabbit's Ear was implanted subcutaneously.

No. of experiment.	Duration of implantation.	Condition of implanted skin at end of period of implantation.		
		Condition of epithelium.	Mitoses in epithelium.	Necrotic epithelium.
	days			
1	7	In part living	Very scanty	Present
2	7	None living	—	,,
3	31	In part living	Very scanty	
4	31	,,	,,	
5	31	,,	,,	
6	31	None living	—	
7	135	Very little living	Absent	

In order to be in a position to interpret some of the appearances found in the area of implantation, a third series of five implantations was made with normal skin of the rabbit's ear, previously kept for two months in absolute alcohol. These experiments will be referred to later.

When actively proliferating epithelium was implanted it was found that, during the first fortnight of implantation, such portions of the basal prickle layer as remained living continued to divide rapidly, so that in the implanted epithelium mitoses were about as numerous as before implantation, while, in addition, loose horny epidermic scales were produced in abundance at the free surface of the epithelium. The circumstance that cell division continued for a time apparently undiminished in implanted actively proliferating epithelium was quite unexpected, for although it is known that normal epithelium preserved in a moist chamber may remain, for as much as a fortnight after removal, living and capable of being grafted on to a healthy granulating surface,* nevertheless such survival seems to be regarded, not unnaturally, as a species of suspended animation, unattended with active growth. Normal epithelium is, however, owing to its relatively slow growth and its scanty amount, unsuitable for determining the effect of implantation on cell activity, while the use of actively proliferating epithelium, on the contrary, enables this effect to be readily ascertained. The proliferating epithelium, after implantation, would appear to possess a restricted supply of nourishment, for it is not possible to recognise capillaries penetrating into the implanted skin, even at the end of the first week. The character of the mitoses in the implanted epithelium is the same as that noted before implantation, both somatic and reduction mitoses being seen, the former predominating, as described and figured in a previous communication.*

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* K. Garrey, "Transplantationen in der Chirurgie," 'Verhandlungen der Gesellschaft Deutscher Naturforscher und Aerzte,' 1906, Erster Teil, S. 205.

Fig. 3.

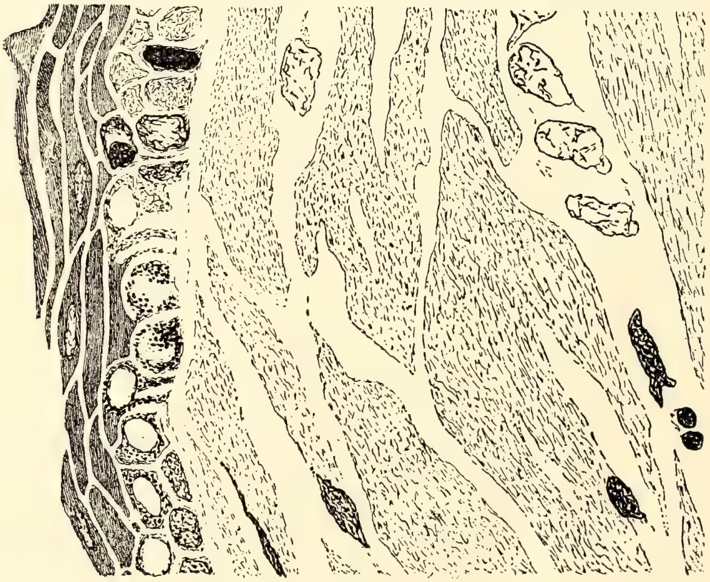

Fig. 4.

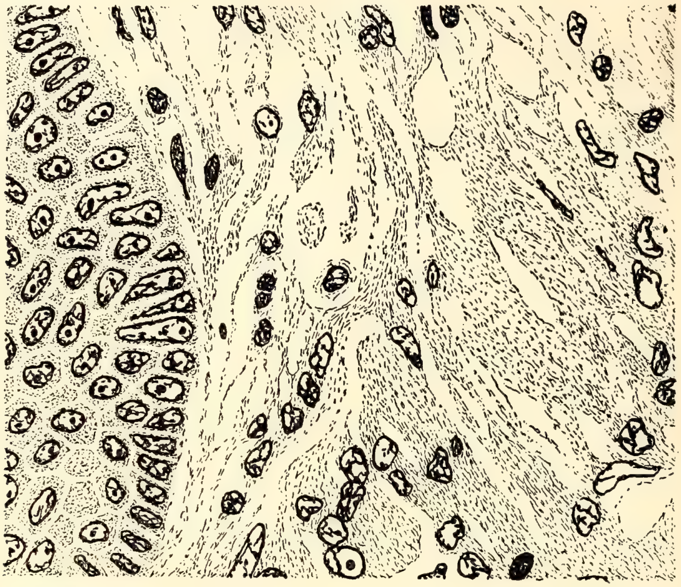

Fig. 3.—Section of normal skin of rabbit's ear, for comparison with the succeeding figure.

Fig. 4.—Section of skin of rabbit's ear, in which a slight degree of epithelial proliferation has been produced by scharlach R. In addition, the nuclei in the corium are increased in number and stain readily; they are not so flattened as in fig. 3.

How long active proliferation continues in implanted skin cannot be definitely determined from the experiments so far made, but it was found that by the 31st day the epithelium was considerably reduced in amount, and that, as in normal skin, division figures were rare. The epithelium still surviving at this period resembled normal surface epithelium, consisting of a flattened expansion of prickle cells, covered with epidermic scales and lying upon a bed of fairly dense connective tissue. No hair follicles or sebaceous glands were seen at this period. Subsequently the epithelium surviving became very scanty in amount.

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* J. O. Wakelin Barratt, "Mitosis in Proliferating Epithelium," 'Roy. Soc. Proc.,' B, vol. 79, 1907, p. 372.

When the normal skin of the rabbit's ear was used for implantation (Table II), after the lapse of about a month, the appearance of the portions implanted was similar to that of the former series at the end of the same period of time.

In implanted skin a variable amount of the prickle layer always died. Polynuclear leucocytes appeared in large numbers about the dead portion, which could be readily recognised by the shrivelled character of its nuclei, which stained imperfectly and diffusely. Not unfrequently dead and living prickle cells lie close together, the latter showing numerous mitoses and spireme figures. Subsequently the polynuclear cells were accompanied, and later, for the most part, replaced by large mononuclear cells with abundant cytoplasm (*cp.* figs. 5 and 7), before which the prickle cells gradually disappeared, so that ultimately the place of the latter was occupied by large mononuclear cells accompanied by multinucleated giant cells, the nuclei of which lay chiefly in the centre of the cell (*cp.* figs. 6, 7, and 8). As in such cases the horny epidermic scales still persisted unaltered, acting like inert bodies, as did also the hair shafts (figs. 7 and 8), it might at first appear as if these large mononucleated cells and giant cell masses were really epithelial cells, which, owing to their changed environment, had undergone metaplasia, losing their prickles and their capacity for undergoing conversion into keratin. That this is not so, however, was shown by the experiments of the third series, in which the implantation of alcohol-hardened skin led to precisely the same appearance of large mononuclear and giant cells, before which the prickle layer disappeared (fig. 8), while the appearance of mononuclear cells was preceded by that of polynuclear leucocytes, just as in the first two series. This action of dead sterile epithelium in attracting polynuclear leucocytes shows that the presence of the latter is not necessarily due to contamination with bacteria. No evidence of the presence of bacteria in the area of implantation could be obtained by staining methods, and the appearance of the site of implantation was that of a purely aseptic process. In this connection it may be observed that, when the normal skin of the rabbit's ear was used for implantation, the same appearance of polynuclear leucocytes at first, and later of mononuclear and giant cells, occurred. Fig. 6

represents a section of a portion of the ear of a healthy rabbit implanted *sub cute* for 31 days (Experiment 3, Table II). It must therefore be concluded that the dead prickle layer exerts a chemiotactic action, which is at first acute, leading to the attraction of polynuclear leucocytes, and, later, becoming less intense, affects mononuclear cells. The source of the latter cells need not be considered here, since the experiments made do not throw any new light upon their mode of origin, beyond the fact that mitoses of the

Fig. 5.

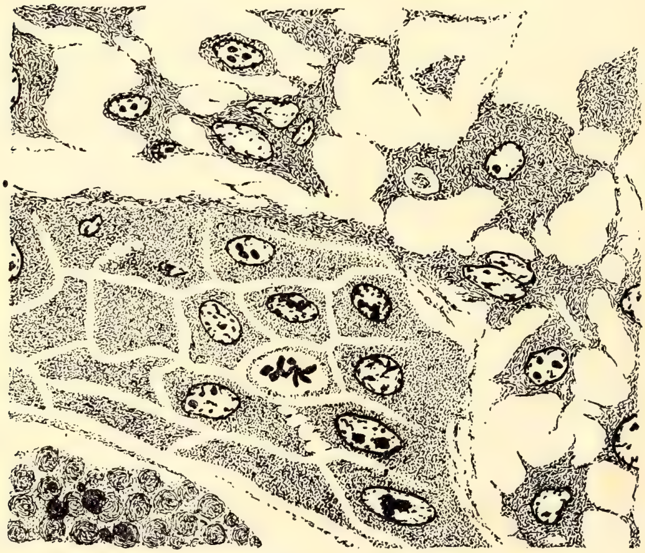

Fig. 6.

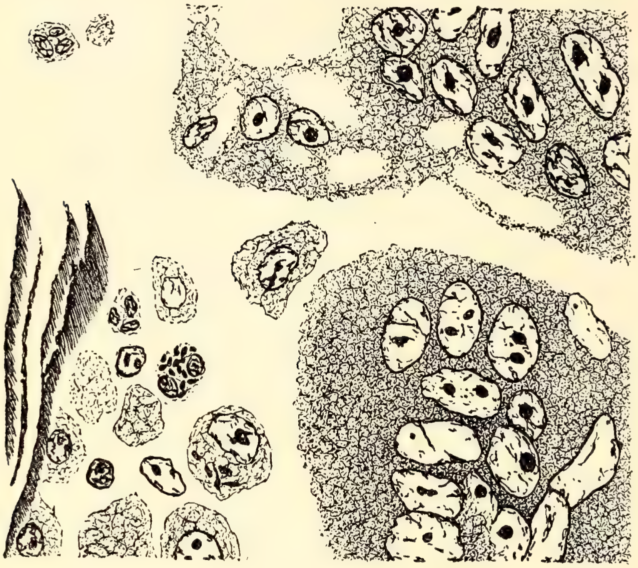

Fig. 5.—Section of skin of rabbit's ear, implanted for seven days under the skin of the back of the rabbit. Previous to implantation, epithelial proliferation had been brought about by means of scharlach R. The prickle cells are still living, and are normal in aspect, one division figure being seen. The nuclei in the corium are surrounded by abundant cytoplasm; well-defined collagen bundles are not present. In contact with the epithelium in the upper left-hand corner necrotic material is seen, consisting of ill-defined spherical masses, among which darkly staining bodies, apparently degenerated nuclei, are found.

Fig. 6.—Section of healthy skin of rabbit's ear, implanted for 31 days under the skin of the back of the rabbit. Above is the stratum corneum unaltered. Below this are seen large mononucleated cells, together with two polynuclear cells, one of which shows basophile granules. Still lower are portions of two large giant cells.

somatic type are frequent among these cells, showing that they multiply *in situ.* The giant cells, which are a striking feature of sections of the area of implantation, do not resemble the giant cells of tubercular lesions, but are like those of myeloid sarcoma, the nuclei being placed centrally. Apart from the *débris* which they exhibit (figs. 5 to 8), the sections recall very vividly the aspect of sarcoma tissue of the round celled variety. Both the giant cells and the single large mononucleated cells exhibit granular cytoplasm, which

Fig. 7.

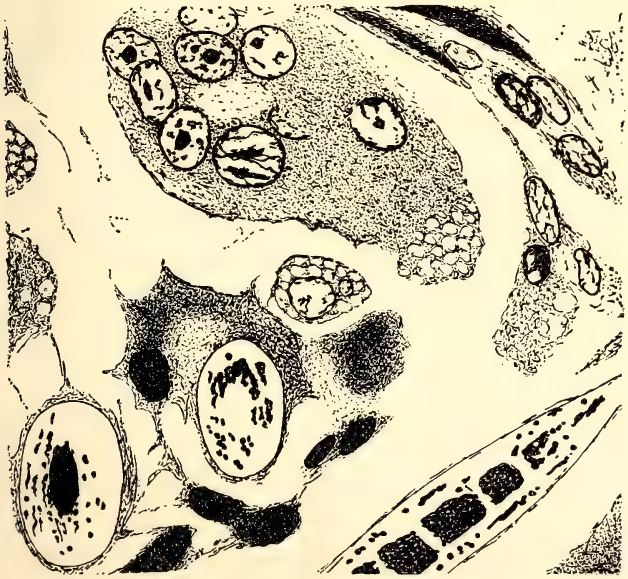

Fig. 8.

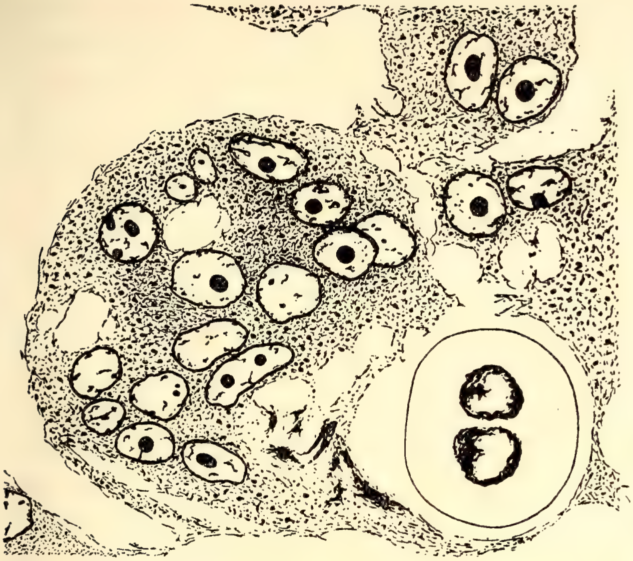

Fig. 7.—Section of nodule resulting from implantation, for 31 days, of skin of rabbit's ear, in which epithelial proliferation had been brought about by injecting scharlach R. No prickle cells are present. Three pigmented hair shafts are seen, two in transverse, one in oblique, section; the latter seems to exhibit slight superficial erosion, the others show no erosion. Mononucleated cells with abundant cytoplasm are seen, and also a giant cell.

Fig. 8.—Section of nodule resulting from implantation of normal skin of the ear of the rabbit for 14 days under the skin of the back of the rabbit. Previous to implantation, the piece of skin employed had been kept in absolute alcohol for two months. An unaltered hair follicle is seen with a giant cell beside and in part surrounding it, together with several large mononucleated cells possessing abundant cytoplasm. This section shows that the cell masses seen in figs. 6 and 7, beneath unaltered stratum corneum or around hair-shafts, cannot be considered as arising by a metaplasia of the implanted epithelial cells.

often contains numerous pigment granules (fig. 8), and sometimes presents a vacuolated character (fig. 7).

It may here be observed that, when epithelial proliferation is produced in the rabbit's ear by the injection of scharlach R (fig. 4), the nuclei of the corium are increased and mononuclear cells rich in cytoplasm become observable. That this is not due to the action of scharlach R, is shown by the fact that repeated attempts to obtain such cell infiltration by injection of this dye, dissolved in olive oil, into the deeper layers of the subcutaneous tissue and also into the testis of the rabbit failed. It follows, therefore, that, as the result of the more active metabolism of the prickle cells under the influence of scharlach R, a chemiotactic action is set up, leading to the appearance of these cells.

The implanted normal corium in the second and third series of experiments did not excite any marked chemiotactic response, no invasion of new cells into it being noted. The cartilage survived in most implantations; when it underwent necrosis, however, it did not become invaded by new cells, its hyaline substance remaining as an inert body, and its nuclei becoming shrivelled and staining indistinctly.

Summary.

1. During the first 7 to 14 days after implantation of actively proliferating epithelium of the rabbit's ear, mitosis proceeds apparently undiminished in amount in such portions of the prickle layer as escape necrosis.

2. In contrast to living prickle cells, dead prickle cells, when implanted under the skin, exert a powerful chemiotactic action, determining at first the appearance of polynuclear cells and later of large mononuclear and giant cells; before the latter the dead prickle layer slowly disappears. Even living prickle cells, when actively proliferating under the influence of scharlach R prior to implantation, induce chemiotactically, through their increased metabolism, the appearance of numerous cells, mostly mononucleated, in the subjacent corium; this is, however, much slighter in degree than that induced by dead proliferated epithelium.

3. No metaplasia of actively proliferating epithelium occurs as a result of implantation.

4. Horny epidermic scales and hair shafts remain intact, behaving as inert bodies.

The Rate of the Assumption of Chloroform by the Blood during Anæsthesia.

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(Communicated by Dr. A. D. Waller, F.R.S. Received March 7,—Read April 18, 1907.)

(From the Physiological Laboratory of the University of London.)

Although it is obvious that the essential condition of anæsthesia is one in which chloroform is associated with the cells of the body, and among these the cells of the central nervous system, the gradual storing-up of the drug must depend upon the supply available in the blood, the red corpuscles of which are, as we have shown, the chief agents for the transport of chloroform either from or to the pulmonary alveoli.

A definite threshold-value for the percentage of chloroform in arterial blood must be reached in order that anæsthesia shall occur, and the state be maintained. The drug, as Tissot, Nicloux, and ourselves have found, is eliminated at first rapidly, and subsequently more slowly, on the cessation of the administration of chloroform. During anæsthesia the drug does not simply accumulate, since the processes of intake and output go on side by side. Though chloroform is eliminated at first with great rapidity, the last traces of the drug take a long time to slowly leak out of the tissues, but apart from this fact the elimination of a high percentage of chloroform in the blood takes place nearly as rapidly, and perhaps at times even as rapidly, as the assumption of a high percentage.

The following scheme probably represents what occurs during anæsthesia:—

- A. Mixture of chloroform vapour and air, constantly supplied in excess.
  - B. Portion which does not reach the alveoli, but is expired.
  - C. Portion which reaches the alveoli and enters the blood.
    - D. Portion which is associated by the tissues.
    - E. Portion which is eliminated at the pulmonary epithelium.

The relative amounts of B and C will, for a given percentage of chloroform, depend upon the rate and depth of the respiratory movements. This is modified as the anæsthetic state progresses to asphyxia, owing to the increasing feebleness of the respiratory centres or to the gradual effect of the drug on the muscles concerned in respiration, a factor which probably is of small importance, since Sherrington has shown that striped muscles, unlike the heart, are not readily poisoned by chloroform. In the case of cats, during the very early stages of anæsthesia by chloroform, the rate of respiration diminishes, and is followed by a marked increase. This variation of respiratory movement would no doubt affect the relative proportions of B and C. Of the chloroform passing inwards by the pulmonary epithelium and which is carried by the blood, part is associated by the cells, D, and a part is eliminated, E. As anæsthesia progresses D will be a decreasing factor, while E will be an increasing factor, owing to the augmentation of chloroform in the blood returning to the lungs. The rate of elimination, E, may be even greater than the rate of assumption, C, though the total amount of C exceed that present in the returning blood. Although the rate at which chloroform is tending to pass out may be greater than the rate at which it tends to pass in, the actual amounts going either way will depend, other things being equal, on the mass of the chloroform on either side of the pulmonary epithelium. If, therefore, the nerve centres remained unaffected, we should expect that with a constant normal type of respiration a condition of equilibrium would be reached between factors A and B and C and E, with any given percentage of chloroform inhaled, which would effect a perfect anæsthesia and not rapidly kill.

During the progress of anæsthesia the nerve centres become paralysed, but not necessarily in a regular order, as we have often noticed that various reflexes vanish or reappear in different sequence in animals of the same species. We should expect, therefore, that the curves indicating the variations of the percentage of chloroform in blood with time would not necessarily be smooth.

The determination of the amount of chloroform which is present in the anæsthetised animal has been attempted in three different ways: (1) By ascertaining the percentage of chloroform in the blood of animals anæsthetised or killed by chloroform. (2) By determination of the amount of chloroform in the blood and various organs of the body *post mortem*.* (3) More frequently by reckoning the difference between the amount of chloroform in the inspired and expired air. Waller,† Vernon Harcourt,‡ Collingwood,§ and

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* Nicloux, 'Comptes Rend.,' 1906, vol. 60, p. 206.

† Waller, 'Lancet,' November 28, 1903.

‡ Harcourt, 'Brit. Med. Journ.,' June, 1906.

§ Collingwood, 'Physiol. Soc. Proc.,' vol. 28, 1905.

Brodie and Widdows* have published the results of work undertaken on the third line, and the last observers have ascertained that the assumption of chloroform is much more rapid in the earlier than in the later stages of anæsthesia. We believe that by this method only a very limited amount of information can be gained as to the physiological processes which are concerned in anæsthesia. With the desire of throwing further light on this question, we have undertaken a number of experiments on the chloroform-content of the blood during the stages of anæsthesia. A very large number of experiments have been made, of which we quote three of the most detailed ones, which are typical of the whole.

Mode of Experiment.

The general mode of the experiment has been described in detail in a former paper.† The method we have now followed is similar, except that nitrous oxide was used instead of ether as the first anæsthetic. The effect of this gas was allowed to pass off and then chloroform was administered. We found some difficulty in maintaining the chloroform-content of the inspired air absolutely constant. The best mode of attaining this is by means of goldbeater's skin bags filled with a definite percentage of chloroform vapour by means of the Dubois apparatus, and this was the method adopted in some of the experiments. The apparatus gives fairly good results within the limits for which it is constructed, *i.e.*, up to percentages of 2, but above this value it is difficult to obtain constantly good results. It is also necessary that the goldbeater's skin bags shall be new and made of fresh skin, as it was found that with old bags, even if these were perfect, the contents became gradually weaker in chloroform, and direct experiments showed that this was due to the absorption of chloroform vapour by the material. With fresh goldbeater's skin this did not occur.‡ All the tubes used in making connection with the bags or cannula in the trachea should also be of wide bore, not less than $\frac{3}{4}$ inch, and rubber junctions should be avoided as far as possible. In other experiments a Woulff's bottle of the following description was used instead of bags. Capacity of the bottle 580 c.c.; volume of chloroform 170 c.c.; surface area 43 sq. cm.; volume of air-space 410 c.c.; diameter of inlet-tube, which was surrounded by a water jacket of the same temperature as the main bath, 2·5 cm.; diameter of outlet-tube 1·5 cm. The bottle was placed in a large bath so that it could be kept at any desired temperature. The apparatus was so arranged that the distance of the lower end of the inlet-tube from the surface of the chloroform could be varied at will. The outlet-tube was connected with the Chauveau-valve apparatus by means of a densimeter bulb 250 c.c. in capacity. By this means the percentage of chloroform in the inspired air could be ascertained. This apparatus proved in many respects satisfactory; not the slightest drag on the respiratory movements was noticed, a matter of great importance in any experiment of this kind, and particularly so when the duration of an experiment is prolonged. We have found that when the normal movements of respiration were mimicked by means of a bellows apparatus, which aspirated a mixture of air and chloroform vapour from the Woulff's bottle, considerable variations, amounting to as much as 50 per cent. of the higher values, were noticed when the rate or amplitude or both were varied. Actual experiments on cats showed that the variations which occur during anæsthesia were much less marked. Thus, during one experiment lasting 20 minutes, the following percentages were obtained: 1·8, 2·1, 2, 1·6, 1·7. In another experiment of 24 minutes' duration, the values at intervals were as follows: 4·1, 3·6, 4·5, 3·1, 2·95, 3·8, 3·75. For the first experiment the average was 1·8 and for the second 3·8.

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* Brodie and Widdows, 'Brit. Med. Journ.,' June, 1906.

† 'Roy. Soc. Proc.,' B, vol. 78, p. 422.

‡ Aspiration of a mixture of chloroform vapour and air from an old goldbeater's skin bag. Weight of chloroform determined densimetrically.

In one experiment the weight of chloroform progressively fell in three-quarters of an hour from 0·045 to 0·032, and with a larger bag the progressive fall was from 0·023 to 0·01 in 20 minutes. 1·1 grammes of the skin of this bag, suspended in air containing chloroform vapour, increased in weight 0·026 gramme, while a similar control in air increased only 0·0015. An experiment with fresh skin showed practically no change in weight. A new bag, 36 inches in circumference, the content of which was analysed at regular intervals, showed no variation in an hour, though a slight decrease in the amount of chloroform took place on prolonged standing.

In the experiment (No. I), asphyxial convulsions began within a minute of the cessation of respiration, and with the continued inhalation of chloroform the convulsions recurred at intervals until 4.23. The heart ceased to beat at 4.28.

The data obtained from this table are given in a graphic form in Curve I, while in Column 7 of the table the type and frequency of the respirations, at the time each sample was taken, are given.

In the experiment (No. II), chloroform was commenced at 2.20 P.M., but before the end of the first minute the animal ceased to breathe. The inhalation was at once stopped, the animal recovered and the experiment restarted at 2.45, by which time the chloroform would have been eliminated. This early and sudden cessation of respiration we have repeatedly noticed with cats, and it is not a mere holding of the breath, as in other experiments it was often found necessary to use artificial respiration in order that the animal should recover. After this has happened, under apparently precisely similar

Experiment I.—In this experiment a Cat weighing 4 kilogrammes was used, and 2-per-cent. chloroform administered by bags. The results obtained are given in the following table. The respiratory movements were recorded by a tambour on the chest.

Time.	Anæsthetic.	Weight of samples of blood taken.	Weight of AgCl found.	Percentage of chlorine.	Differences in percentage of chlorine, reckoned as $CHCl_3$.	Type and frequency of respirations. Number per minute.	Remarks.
1.20	N_2O						
1.40	,,	3·2787	0·0448	0·3378	—	—	Control sample
1.55	$CHCl_3$, 2 per cent.	—	—	—	—	Average depth; 84	
1.56	,,	2·5603	0·0355	0·3428	0·0056		
1.58	,,	2·8968	0·0404	0·3448	0·0078	Twice as deep; 61	
2.1	,,	2·9232	0·0416	0·35186	0·0157	Depth as above; 63	
2.6	,,	2·6256	0·0377	0·355	0·0193	Below average; 45	Reflexes (eye) just gone
2.11	,,	2·6288	0·0388	0·3649	0·03	Average depth; 57	
2.25	,,	3·4286	0·0499	0·3598	0·0247	,, 57	
2.35	,,	2·9925	0·0444	0·3668	0·0325	Below average; 95	Stepping movements began
2.45	,,	2·6117	0·04	0·3787	0·0458		
2.55	,,	2·7168	0·044	0·364	0·0294	Average depth; 63	
3.10	,,	2·8223	0·0429	0·3758	0·0426	,, 56	
						During this time average 69	
4.15	,,	3·7013	0·0574	0·3834	0·0512	,, 49 to 34	Respiration ceased

Experiment II.—Weight of Cat, 3·2 kilogrammes. Chloroform administered by a Woulff's bottle. Percentage of chloroform vapour about 2 per cent. Respiratory movements recorded as in Experiment I.

Time, in minutes.	Anæsthetic.	Weight of samples of blood.	Weight of AgCl found.	Percentage of chlorine.	Differences in percentages of chlorine, reckoned as $CHCl_3$.	Type and frequency of respirations per minute.	Remarks.
—	N_2O	3·4226	0·0473	0·3417	—	—	Control sample
0	$CHCl_3$, 2 per cent. on						
1	,, ,,	2·3853	0·0341	0·3535	0·0132	Slightly above normal; 180	
3	,, ,,	3·4803	0·0512	0·3637	0·0247	Average depth; 91	
4	,, ,,	—	—	—	—	—	Eye reflexes gone
6	,, ,,	3·2651	0·0479	0·3627	0·0236	Slightly below average; 77	
10	,, ,,	3·2084	0·0483	0·3722	0·0342	Average depth; 97	
15	,, ,,	3·5437	0·0518	0·3614	0·022	,, 110	
20	,, ,,	2·9433	0·044	0·3696	0·0313	,, 66	
30	,, ,,	3·0934	0·0463	0·37	0·0318	,, 92	
40	,, ,,	3·1379	0.0486	0·3829	0·0463	,, 107	
50	,, ,,	3·3724	0·0513	0·376	0·0386	,, 107	
75	,, ,,	—	—	—	—	Constant depth and frequency	
81	,, ,,	2·8224	0·0438	0·3837	0·047	Average depth; 96	
93·3	,, ,,	4·6549	0·0729	0·3872	0·0511	—	Respiration ceased at 93; blood difficult to get; animal squeezed
95	,, ,,	—	—	—	—	—	Asphyxial convulsion

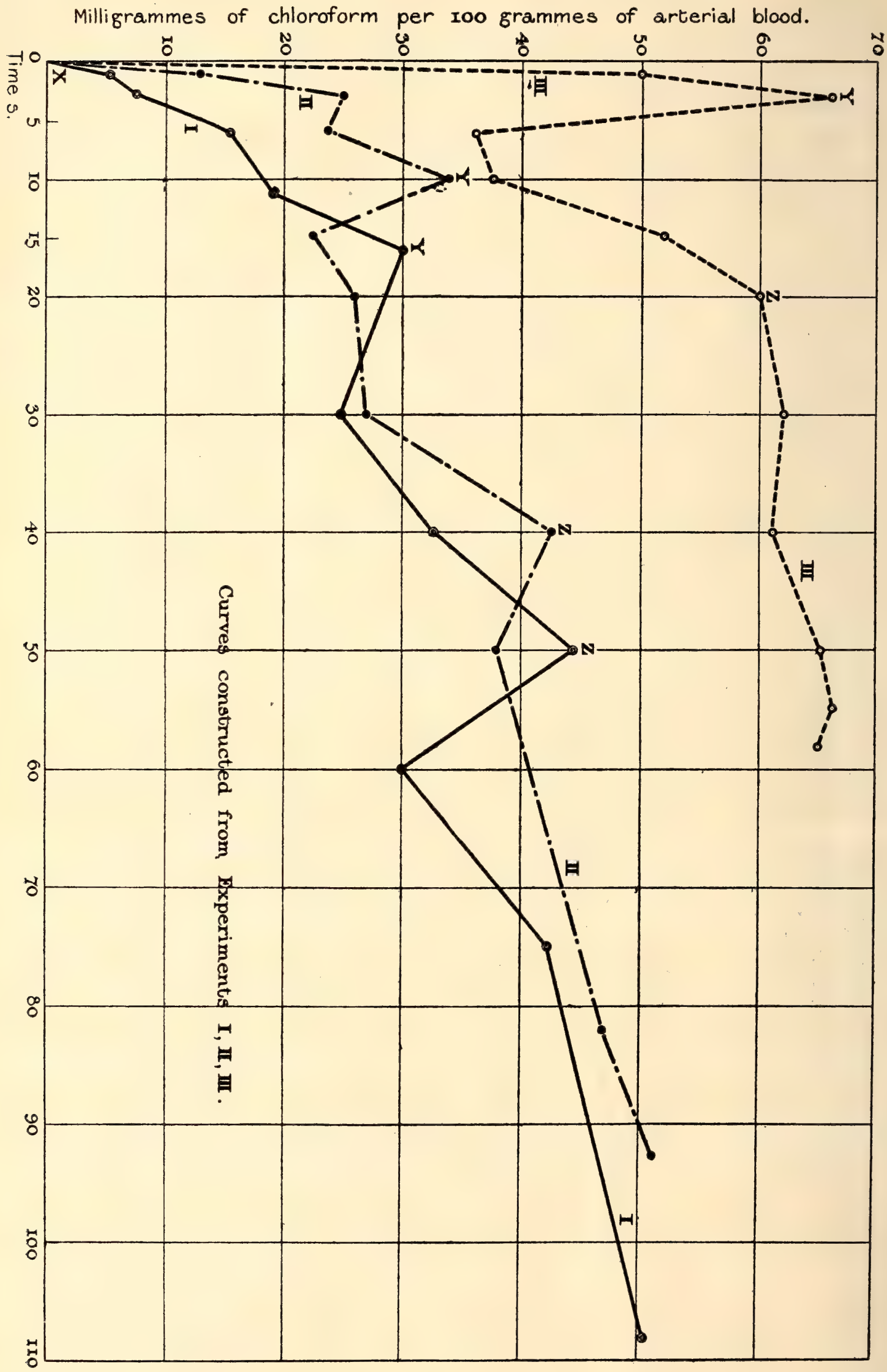
conditions, with the same percentage of chloroform the subsequent anæsthetisation will generally proceed in a perfectly normal manner for a prolonged period of time. We consider this is a fact of much importance which will be subsequently considered.

The results of this experiment are shown in Curve II. The type and frequency of the respirations at the times the samples were taken are given in Column 7 of the table.

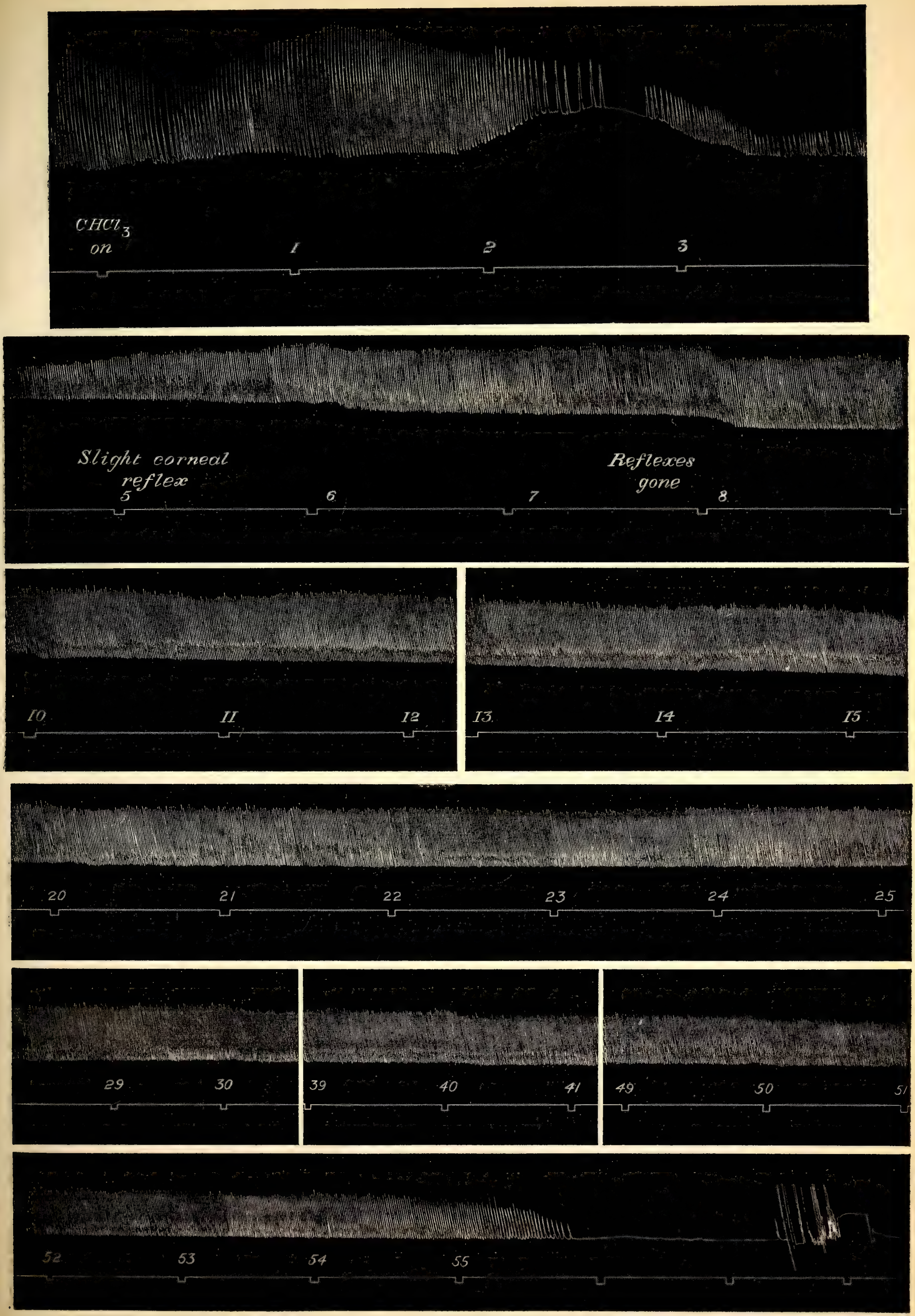
Experiment III.—Cat. Weight 3·5 kilogrammes. Chloroform, between 3 and 4 per cent., administered by a Woulff's bottle. This experiment is of a special interest, as the early cessation of respiration mentioned in connection with the last experiment took place, but the animal recovered naturally, without it being necessary to discontinue the chloroform.

Time.	Anæsthetic.	Weight of sample of blood taken.	Weight of AgCl found.	Percentage of chlorine.	Differences in percentages of chlorine, reckoned as $CHCl_3$.	Remarks.
12. 15	N_2O on					
12. 32	,,	3 ·3454	0 ·0456	0 ·337	—	Control
12. 40	Chloroform 3–4 per cent.					
12. 41	,,	2 ·444	0 ·0377	0 ·3814	0 ·0498	
12. 43	,,	2 ·4221	0 ·0388	0 ·3961	0 ·066	
2. 44	,,	—	—	—	—	Slight eye reflexes
2. 46	,,	3 ·3243	0 ·0497	0 ·3696	0 ·0366	
2. 48	,,	—	—	—	—	Reflexes gone
2. 50	,,	2 ·4079	0 ·0361	0 ·3707	0 ·0378	
2. 55	,,	2 ·8451	0·0441	0 ·3832	0 ·0519	
1. 0	,,	2 ·954	0 ·0467	0 ·3909	0 ·0604	
1. 10	,,	2 ·7349	0 ·0434	0 ·3923	0 ·0621	
1. 20	,,	2 ·7826	0 ·0441	0 ·3918	0 ·0615	
1. 30	,,	2 ·9142	0 ·0466	0 ·3954	0 ·0655	
1. 35½	,,	2 ·4282	0 ·0389	0 ·3961	0 ·0663	Respiration ceasing
1. 38	,,	1 ·8758	0 ·03	0 ·3954	0 ·0656	1st asphyxial convulsion

In Curve III the results of this experiment are illustrated graphically, and we append a portion of the respiration tracing:—



Curves constructed from Experiments I, II, III.



Respiratory Tracing of Experiment III.

Conclusions.

From the tables and curves that have been given, we consider that the views expressed in the earlier part of the paper, which were based on a much larger number of experiments undertaken with various other objects, and with which this paper does not deal, are justified. The chloroform-content of the blood rises in the initial stages of anæsthesia with great rapidity to a value which approaches a maximum. During this period the quantity of chloroform in the blood appears to affect particularly the respiratory centres, so that breathing becomes slower and often ceases during the first few minutes of anæsthesia, and it is necessary to resort to artificial respiration in order to prevent the animal dying. With the percentages of chloroform that have been employed, we have never noticed failure of the heart at this stage. The cessation or slowing of respiration at this stage is, as might have been expected, more liable to occur with high than with low percentages of chloroform. Sudden failure of the heart's action has never been noticed by us in cats under the conditions of our experiments, where no percentages of chloroform above 5 have been employed. We have found that a definite danger-point occurs in the first few minutes of anæsthesia, owing to paralysis of the respiratory nervous mechanism.

If the animal naturally passes this stage, which is marked on the curves between X and Y, as in Experiment III, or is restored either by stopping the chloroform inhalation or by artificial respiration, then on continuing the anæsthetic the amount of chloroform in the blood quickly rises again towards a maximum value. An equilibrium between the factors which determine the amount of chloroform in the blood subsequently appears to be obtained, the processes of intake and output at the surface of the lung going on side by side. This stage extends from point Z onwards.

This state of equilibrium is reached, and may persist for a considerable length of time. The exact length of this period differs with different individuals, and even in the same individual in different experiments. During this period the animal may die at any moment should any disturbing influences come into play, and therefore the state is not one of safety. During this period the animal can always be killed with chloroform, even though the percentage of chloroform in the blood rises only very slowly. We would emphasise the point that the difference between the amount of chloroform in the blood throughout this anæsthetic stage and death is very minute. It will be noticed that the difference between the percentages of chloroform in the blood at the point where the corneal reflexes vanish and the maximum values attained are by no means large in actual value, but are relatively so, averaging, in the experiments quoted, about 50 per cent. After the corneal reflexes have vanished, even though the percentage of chloroform administered be kept absolutely constant, there is no criterion other than analysis by which we can judge of the progress of anæsthesia in these animals.

In our experiments, death always occurred by failure of the respiration succeeded by asphyxia. Under the conditions of our experiments undertaken on a very large number of cats, we have never noticed death by heart failure preceding cessation of respiration.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work; and also our appreciation of the valuable assistance of Mr. G. W. Ellis, who has helped in a large number of the laborious analyses.

Function of the Red Corpuscles in Chloroform Anæsthesia.

By George A. Buckmaster, Assistant Professor of Physiology, University College, University of London, and J. A. Gardner, Lecturer on Physiological Chemistry, University of London.

(Communicated by Dr. A. D. Waller, F.R.S. Received March 12,—Read April 18, 1907.)

(From the Physiological Laboratory of the University of London.)

It has been conclusively shown by Pohl,* Nicloux,† Moore and Roaf,‡ and ourselves§ that when chloroform is associated with the blood, the bulk of the drug is held by the red corpuscles. It would appear, therefore, highly probable that in chloroform anæsthesia the transport from and to the surface of the lungs is a function of the red corpuscles. In order to throw more light on this point, we have investigated the effect of the variations in the volume of blood in circulation on the duration of anæsthesia and on the percentage of chloroform in the blood.

Removal of blood, other conditions being constant, should not alter the percentage of chloroform in the blood. Several causes might separately or together prevent the conditions being constant, and therefore bring about deviations from the above conclusion.

1. The gradual entrance into the blood of extra-vascular liquid. According to Starling,‖ "the fluids contained in the tissue spaces possess the same tonicity and the same composition in salts as the blood-plasma." If, therefore, the extra-vascular fluids entered the blood-stream instantly to an amount equal to that of the blood withdrawn, the percentage of chlorine in the blood would only be slightly raised, because the corpuscles contain a somewhat lower percentage of chlorine than the plasma.¶ The restoration of the volume after hæmorrhage is known to be a gradual process, and during the time and under the conditions of our experiments could not have taken place to any marked extent. Therefore the variations in the chlorine-content of the blood would be practically negligible, and well within the errors of experiment. This conclusion was repeatedly confirmed in the course of our experiments by analyses made at the beginning, before and after bleeding.

2. An entrance of red corpuscles into the blood-stream, consequent on

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* 'Archiv f. exp. Path. u. Pharmak.,' vol. 28, 1890—1891.

† 'Comptes Rendus,' Nos. 2, 3, 7, 1906.

‡ 'Roy. Soc. Proc.,' vol. 73, 1904.

§ 'Roy. Soc. Proc.,' B, vol. 78, 1906.

‖ 'Journal of Physiology,' vol. 19, No. 4, 1896.

¶ Buckmaster and Gardner, 'Roy. Soc. Proc.,' B, vol. 78, 1906.

hæmorrhage, is known to be a very slow process, and one dependent upon the activity of the bone-marrow. The evidence which exists on this point, therefore, negatives the idea that any appreciable effect on the chlorine-content of the blood can be due to this cause.

3. Abnormal depth and frequency of respiration, combined with a high percentage of chloroform, might conceivably cause abnormality in any set of parallel experiments made before and after bleeding by the chloroform entering the plasma to an excessive amount.* The possibility of this factor coming into play necessitated a large number of experiments.

Finally, the peculiar nature of the anæsthetic process, to which we have drawn attention in another paper,† as an examination of the published curves will at once make clear, renders it unlikely that any definite conclusions could be drawn from a small number of experiments as to the effect produced by alteration in the volume of circulating blood. In the paper referred to we showed that the percentage of chloroform in blood rapidly rises to a point approaching a maximal value. When this point is reached, which may occur within the first few minutes of an experiment, the animal often ceases to breathe. This we have alluded to as the first danger-point of anæsthesia. When this point is passed naturally, the chlorine-content of the blood falls, but again rapidly rises. In most experiments, when breathing stopped at this point, we found it better to allow the animal to resume breathing and restart the experiment. In either case the maximal value was quickly reached again, when an equilibrium occurred between the intake and output of the drug. This state of equilibrium, when the percentage of chloroform only rose very slowly, was often maintained for a considerable period of time. During this period the respiration may stop at any moment from slight causes which cannot be controlled. This further rendered it necessary to make a large number of experiments.

The operative procedures for the administration of chloroform and for withdrawing blood were identical with those described in former papers.

Experiments in which the Asphyxial State was rapidly reached.

The percentage of chloroform used in these experiments was above 3 per cent. and under 5 per cent. Sometimes bags were used, sometimes a Woulff's bottle such as we have employed in experiments previously described, so that a slight drag on the respiratory movements was produced. These experiments may be fairly taken as representative of those in which death occurred at the first danger-point of anæsthesia.

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* Buckmaster and Gardner, *ibid.*

† 'Roy. Soc. Proc.,' this volume.

The general plan of the experiment was to anæsthetise the animal with ether or nitrous oxide, allow the anæsthetic to be disengaged from the body, and then administer a known percentage of chloroform. The determination of the chloroform-content of the blood was made at the asphyxial point. The animal was then allowed to recover from the full effect of the chloroform, either naturally or by artificial respiration, and sufficient time was allowed for practically all the chloroform to be eliminated from the blood. One hour to one and a-half hours was usually sufficient for this, and it was generally necessary to give the animal a little ether or nitrous oxide in order to preserve unconsciousness. During this period the animal was bled from the carotid artery to a known amount. The quantity of blood withdrawn varied with the weight of the animal. Sometimes the whole amount was taken at once, but generally in successive small quantities. The animal was re-anæsthetised and another determination of the amount of chloroform in the blood made at the asphyxial point, but before re-anæsthetisation a sample of blood was always taken as a control. The details of the experiments are recorded in Table I.

These are the only experiments in which the respiration-curves definitely indicated that asphyxia occurred both before and after bleeding in the initial stage of anæsthesia. In five of the seven experiments, comparative determinations were made before and after bleeding. Of these, in two cases the percentages of chloroform found in the blood were practically identical. In two cases the percentage was higher after than before bleeding, and in one case lower. In the other two experiments in which values after bleeding were obtained, the figures are fairly normal.

Before bleeding the average percentage of chloroform in 100 grammes of blood was approximately 0·043 gramme and after bleeding 0·045 gramme. It would appear, therefore, that in the above experiments the percentage of chloroform in the blood is not altered by bleeding.

Experiments in which Asphyxia took place during the Second Stage of Anæsthesia.

In these experiments chloroform was inhaled from the Woulff's bottle described at length in a former paper "On the Rate of the Assumption of Chloroform by the Blood during Anæsthesia." In those cases where respiration stopped within the first few minutes, the animal was brought round artificially or allowed to recover naturally and the anæsthetic again administered continuously. In other respects the procedure was the same as in the first group of experiments. The details of the experiments are recorded in Table II.

Table I.—*Experiments in which the Asphyxial state was rapidly reached.*

No. of experiment.	Weight of cat in kilos.	First anæsthetic.	Percentage of CHCl$_3$ inhaled.	Chloroform apparatus.	Time to asphyxia in minutes.		Amount of blood withdrawn in c.c.	Percentage of chloroform in blood at asphyxia in grammes per 100 grammes blood.		Remarks.
					Before bleeding.	After bleeding.		Before bleeding.	After bleeding.	
I	2·6	Ether	3—4	Bag	14	10	18	0·053	0·063	Respiration curves show asphyxia at first danger-point. Asphyxial convulsions after bleeding at 12 minutes
II	3·3	Ether	3—4	Bag	—	11	30	—	0·037	Animal bled before experiment
III	2·4	Ether	4—5	Bag	4·5	4	13·5	—	0·0412	
			4—5	”	9	2·5	—	—	—	Re-done
			4—5	”	—	3	—	—	—	Re-done half hour later
			4—5	”	—	3	—	—	—	Re-done 8 minutes later
			4—5	”	—	3·5	—	—	—	Re-done 10 ”
IV	3·2	Ether	5	Bag	29	20	18	0·0404	—	Sample before bleeding at asphyxial convulsions
			5	”	—	16—17	—	—	0·0413	Re-done after 1 hour
			5	”	—	16—17	—	—	—	Re-done 40 minutes later
						17	—	—	—	Re-done 30 ”
V	2·3	Ether	4·5	Bag	11					
			4·5	”	14	11	19	0·0369	—	Re-done 30 ”
			4·5	”	—	13	2	—	0·0369	Re-done 20 ”
			4·5	”	—	10	—	—	—	Re-done 20 ”
			4·5	”	—	12·5	—	—	—	Re-done 30 ”
VI	2·2	Ether	3—4	Bag	4·5 3					
			3—4	”	—	—	—	0·0246	—	Re-done 10 minutes later; breathing shallow and abnormal after the first minute
							20			
			3—4	”	—	9				
			3—4	”	—	15	—	—	0·048	Re-done 1 hour later
VII	2·3	N$_2$O	3·5—4·5	Bottle	8					
					9	—	—	0·0605	—	Re-done 28 minutes later
							25			
						4	—	—	0·0488	Blood proved free from CHCl$_3$ before re-anæsthetisation

Table II.—*Experiments in which Asphyxia occurred during the Second Stage of Anæsthesia.*

No. of experiment.	Weight of cat in kilos.	First anæsthetic.	Percentage of $CHCl_3$ inspired.	Apparatus.	Time in minutes.		Blood withdrawn in c.c.	Percentage of chloroform in grammes per 100 grammes blood.		Remarks.
					Before bleeding.	After bleeding.		Before bleeding.	After bleeding.	
VIII	3·6	N_2O	3·5	Bottle	63	—	—	0·0499	—	Initial stage passed naturally, though respiration almost ceased
			3·5	,,	—	—	30	—	—	After interval of 1 hour, analysis showed blood free from chloroform
			3·5	,,	—	29—30	—	—	0·05	Sample from heart
IX	3·6	N_2O	4	Bottle	31	—	—	0·068	—	Asphyxial convulsions at time of sample. Rhythmical movement of hind limbs and tail during anæsthesia
			4	,,	—	18	30 —	—	0·0438	Sample from heart and body cavity
X	3·8	N_2O	3·5	Bottle	24	—	—	0·048	—	Stepping movements occurred
							35	—	—	Sample proved blood free from $CHCl_3$
			3·5	,,	—	20	—	—	0·052	Stepping movements occurred
XI	3	N_2O	3	Bottle	30	—	—	0·0476	—	$CHCl_3$ discontinued for short time after first minute. Time commences from re-anæsthetisation
				,,	—	53	26 —	—	0·052	Stepping movements more marked than before

XII	3·5	N_2O	2—2·5	Bottle	75	—	— 34	0·0532	—	Initial stage passed naturally, was well shown on tracing
						108	—	—	0·0489	After recovery from chloroform animal slept for some time. Analysis showed all $CHCl_3$ out of blood. No stepping movements
XIII	2·8	N_2O	2—2·5	Bottle	—	—	—	—	—	Initial stage recovered from naturally
								0·0405	—	30 minutes after commencement of $CHCl_3$
								0·0464	—	15 minutes later
					80	—	—	0·0482	—	Cessation of respiration. Slept after recovery
							32	—	—	Sample taken 80 minutes later showed only 0·001 chloroform
			2·5	”	—	69	—	—	0·0548	Initial process passed naturally, subsequently slight stepping movements. First danger-point well marked
XIV	4	N_2O	2—2·5	Bottle	—	—	—	0·043	—	After 30 minutes from commencement of chloroform
					41	—	—	0·0355	—	11 minutes later, on cessation of respiration, which occurred very gradually
							39			1½ hours' elapse
			2·5	”	—	—	—	—	—	Initial danger-point well marked
						55	—	—	0·0467	At asphyxia
										Experiment absolutely uncomplicated

It will be seen, therefore, that the average percentage of chloroform in the blood at the asphyxial point is 0·048 before and 0·051 after bleeding, or eliminating the first two figures in Column 9, quoted in Experiment XIII, and the first figure in same column in Experiment XIV, as these values were determined in samples taken considerably before asphyxia, the corrected averages are 0·05 before and 0·0497 after bleeding. In one experiment the figures were sensibly the same before and after withdrawal of blood, and in four experiments there was a slight increase after the withdrawal of blood. In the two remaining experiments a decrease was observed after a hæmorrhage.

In the following table (No. III), we quote two experiments in which the respiration tracings showed that asphyxia took place before bleeding in the second stage of anæsthesia, and after bleeding at the initial stage.

Experiments in which comparisons were made of the percentage of chloroform in the blood at the asphyxial stage after hæmorrhage and after the replacement of the blood which had been removed by the blood of another animal of the same species. All the observations were made in the second stage of anæsthesia.

The replacement of blood was effected in the following way:—A cat was anæsthetised with ether and a cannula placed in the carotid artery. The cannula was connected by a short rubber tube fitted with a clip, with the outflow end of a burette. This was warmed and smeared inside with olive oil, and before making connection with the cannula, the cannula, rubber tube and about an inch of the burette were filled with warm olive oil, so that the blood could be received from the animal without coming in contact with the air or glass, and without cooling to any serious extent. A small cannula had been previously placed in the femoral vein of the animal which was the subject of the anæsthetic experiment. This cannula was filled with a warm solution of sodium sulphate of a strength isotonic with that of blood plasma. The burette was now disconnected from the cannula in the carotid artery, and connected with the cannula in the femoral vein, care being taken that no air entered the circulation. The requisite amount of blood was then allowed slowly to flow during the course of two or three minutes into the animal. The whole transfer of blood was effected in less than four minutes. In none of our experiments did we notice any disturbing effects produced by the introduction of the blood. The animal was then re-anæsthetised with chloroform, after recovering from the first anæsthetic, precisely as in the first part of the experiment. Details of these experiments are recorded in Table IV.

In two out of the three complete experiments the percentage of chloroform in the blood at the asphyxial point after bleeding and after replacement

Table III.—*Asphyxia during the Second Stage of Anæsthesia before and during the First Stage after Bleeding.*

No. of experiment.	Weight of cat in kilos.	First anæsthetic.	Percentage of $CHCl_3$.	Apparatus.	Time in minutes.		Blood withdrawn in c.c.	Percentage of chloroform in grammes per 100 grammes of blood.		Remarks.
					Before bleeding.	After bleeding.		Before bleeding.	After bleeding.	
XV	2·6	N_2O	4·5	Bag	20	—	—	0·0409	—	Sample taken during asphyxial convulsions. Initial stage well shown in respiration curve
							18·5			85 minutes elapse so that chloroform may be eliminated
			4·5	Bag	—	8—9	—	—	0·0298	Respiration rapid and deep at first, and ceased between first and second stage
XVI	3·5	N_2O	4	Bag	30	—	—	0·0484	—	Second stage reached naturally, as shown by tracing. Stepping movements
							29			80 minutes elapse for chloroform to be eliminated
			4	"	—	5	—	—	0·0776	Respiration deep and rapid, and tracing clearly showed that asphyxia occurred at initial stage. One hour elapses for $CHCl_3$ to be eliminated
			4·5	"	—	3·5	—	—	0·0504	Asphyxia at initial stage, shown in tracing

Table IV.—*Experiments giving the Percentage of Chloroform in Blood at Asphyxial Stage, after Hæmorrhage and after Replacement of Blood.*

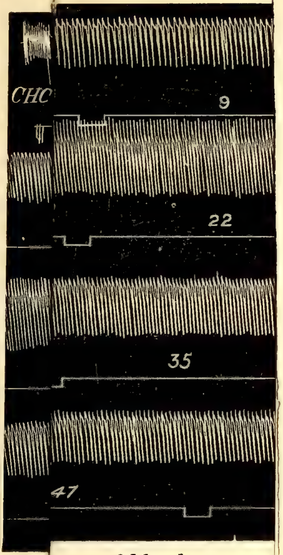
No. of experiment.	Weight of cat in kilos.	First anæsthetic.	Percentage of chloroform inhaled.	Apparatus.	Time in minutes.		Blood withdrawn in c.c.	Blood restored in c.c.	Percentage of chloroform in grammes per 100 grammes of blood		Remarks.
					After bleeding.	After restoration of blood.			After bleeding.	After restoration of blood.	
XVII	3·3	N_2O	—	—	—	—	32				
			2—3	Bottle	104	—	—	—	lost	—	Cessation of respiration in initial stage, and animal allowed to recover for half an hour. Time starts from this point. Stepping movements of hind limbs well marked
								38			80 minutes elapse to allow chloroform to be eliminated
			2—3	,,	—	19	—	—	—	0·061	
											70 minutes elapse and experiment repeated
						54	—	—	—	0·0632	
XVIII	4·0	N_2O	—	—	—	—	32				
			2	Bottle	126	—	—	—	0·0421	—	Animal ceased to breathe at initial stage, but was recovered by artificial respiration. Time starts from this point
								42			
			2	,,	—	153	—	—	—	0·0426	

XIX	3·5	N_2O	—	—	—	—	40				
			2	Bottle	78	—	—	—	0·0437	—	Initial stage passed naturally. Stepping movements. Asphyxia very sudden
								40			70 minutes elapse
			2	,,	—	103	—	—	—	0·0518	Breathing shallow. Initial stage passed naturally
XX	3·6	N_2O	—	—	—	—	32				
			3·5—4	Bottle	44	—	—	—	lost	—	Initial stage passed naturally
								38			
			3·5—4	,,	—	55	—	—	—	0·057	
XXI	3·2	N_2O	—	—	—	—	24				
			4—5	Bottle	52	—	—	—	0·0768	—	Animal ceased to breathe at initial stage. Artificial respiration
								30			90 minutes elapse
			4—5	—	—	52	—	—	—	0·0768	

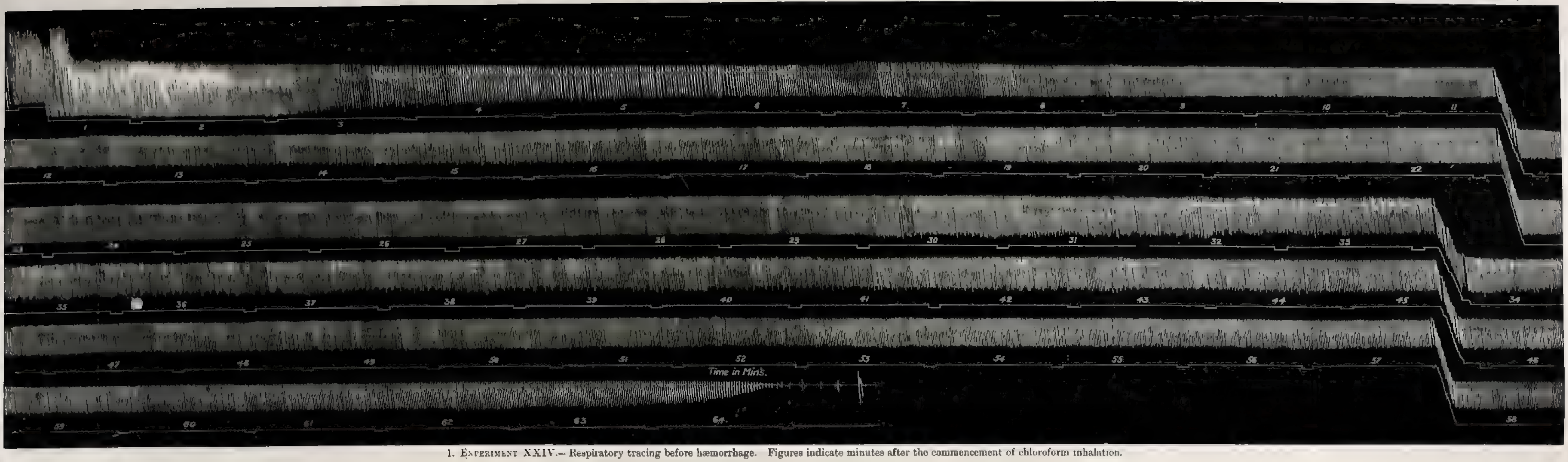
Table V.

No. of experiment.	Weight of cat in kilos.	First anæsthetic.	Percentage of $CHCl_3$ inhaled.	Apparatus.	Time in minutes.			Blood withdrawn in c.c.	Blood replaced in c.c.	Chloroform in grammes per 100 grammes of blood.			Remarks.
					Before bleeding.	After bleeding.	After replacement of blood.			Before bleeding.	After bleeding.	After replacement of blood.	
XXII	3·1	N_2O	3	Bottle	19—20	—	—	— 27	—	0·0563			1 hour to recover. Sample showed chloroform had been completely eliminated
			4	,,	—	12	—	—	—	—	0·008*	—	*Respiration curve indicated that the animal had passed the first danger-point but respired so badly that the experiment was stopped and inhalation stopped. Blood obtained with difficulty and animal took a number of rapid deep respirations before the blood was obtained. Hence the low percentage of chloroform
			3—4	,,	—	—	11	—	30 —	—	—	0·02	Cheyne Stokes' respiration. Stepping movements. Respiration ceased at first stage, but revived naturally on stopping the anæsthetic
			4	,,	—	—	8	—	—	—	—	0·0365	After 40 minutes' interval re-anæsthetised by $CHCl_3$. After respiration ceased, syncope. Sample from chest cavity
XXIII	3·1	N_2O	2	Bag	39	—	—	— 26	—	0·0376	—	—	Transition from first to second stage scarcely noticeable in respiration tracing. No stepping movements
			2	,,	—	13	—	—	—	—	0·028	—	Sample 100 minutes after asphyxiation showed traces of $CHCl_3$. Next experiment 22 minutes afterwards. Respirations very deep and rapid and ceased before second stage had fairly begun
						4	—	—	—	—	not done	—	Experiment re-done after 20 minutes. Cessation of respiration at first stage. Animal recovered artificially and we decided to proceed with third portion of experiment. Pupils not dilated in the least in the last two experiments
			2	,,	—	—	41	—	41 —	—	—	0·035	Interval of 1 hour elapses. Transition from stage 1 to 2 scarcely noticeable
XXIV	3·4	N_2O	3	Bottle	64	—	—	— 30	—	0·0442	—	—	No complications observed. First stage passed naturally. Transition well seen on tracing
			2·5—3	,,	—	76	—	—	—	—	0·0598	—	Interval 75 minutes. Blood found free of chloroform. First stage passed naturally. Transition well shown on tracing
			3	,,	—	—	49	—	40 —	—	—	0·0508	After replacement of blood, transition passed naturally, well shown in tracing
XXV†	4·6	N_2O	3	Bottle	119	—	—	— 38	—	0·061	—	—	Transition stage not well marked. No complications
			3	,,	—	31	—	—	— 50	—	0·0597	—	Interval 76 minutes for recovery. Sample showed that blood still contained a little $CHCl_3$. Tracing showed second stage was reached
			4	,,	—	—	34	—	—	—	—	0·0489	Interval of 80 minutes, during which animal slept for almost entire time. No N_2O required. Transition stage not well marked in this experiment

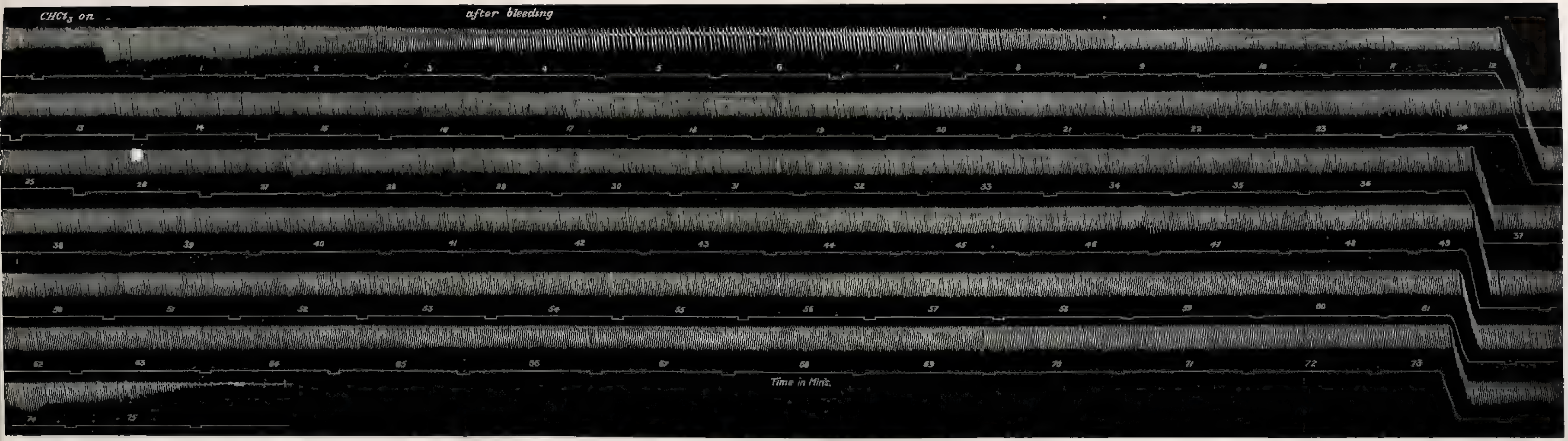
† In this experiment both animals were injected with hirudin before chloroform anæsthesia. Each animal received 0·03 gramme of hirudin dissolved in 2·5 c.c. of isotonic sodium sulphate. This amount removed any tendency to clotting in the cannula and greatly facilitated the removal of samples of blood.



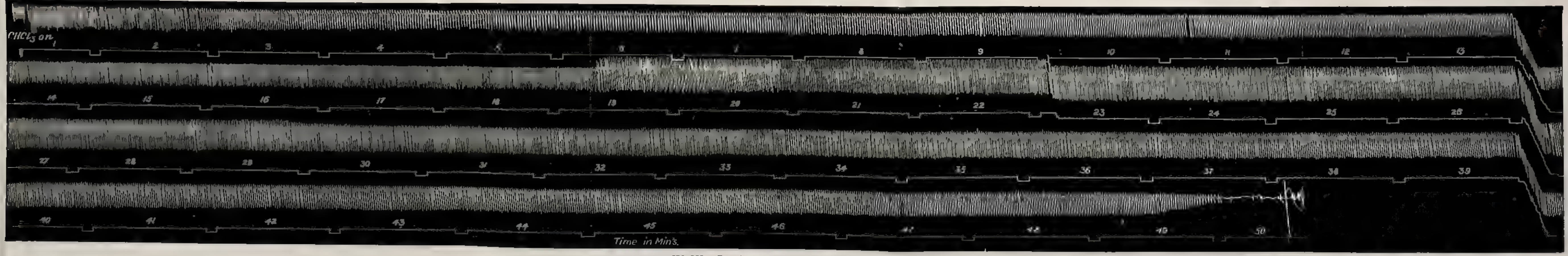
ment of blood.



1. Experiment XXIV.—Respiratory tracing before hæmorrhage. Figures indicate minutes after the commencement of chloroform inhalation.



2. Experiment XXIV.—Respiratory tracing taken after hæmorrhage.



3. Experiment XXIV.—Respiratory tracing taken after replacement of blood.

of blood is practically the same. In the third experiment a small increase is noticed after restoration of blood. In the case of the two incomplete experiments the percentages of chloroform in the blood after replacement are of the same order of magnitude as is found in normal animals at the stage when respiration ceases.

Experiments in which comparisons were made of the percentage of chloroform in the blood at the asphyxial state in the normal animal, after a hæmorrhage, and after the replacement of the blood which had been withdrawn by the blood of another animal of the same species.

The mode of experiment was the same as that already described; the results are given in table (No. V).

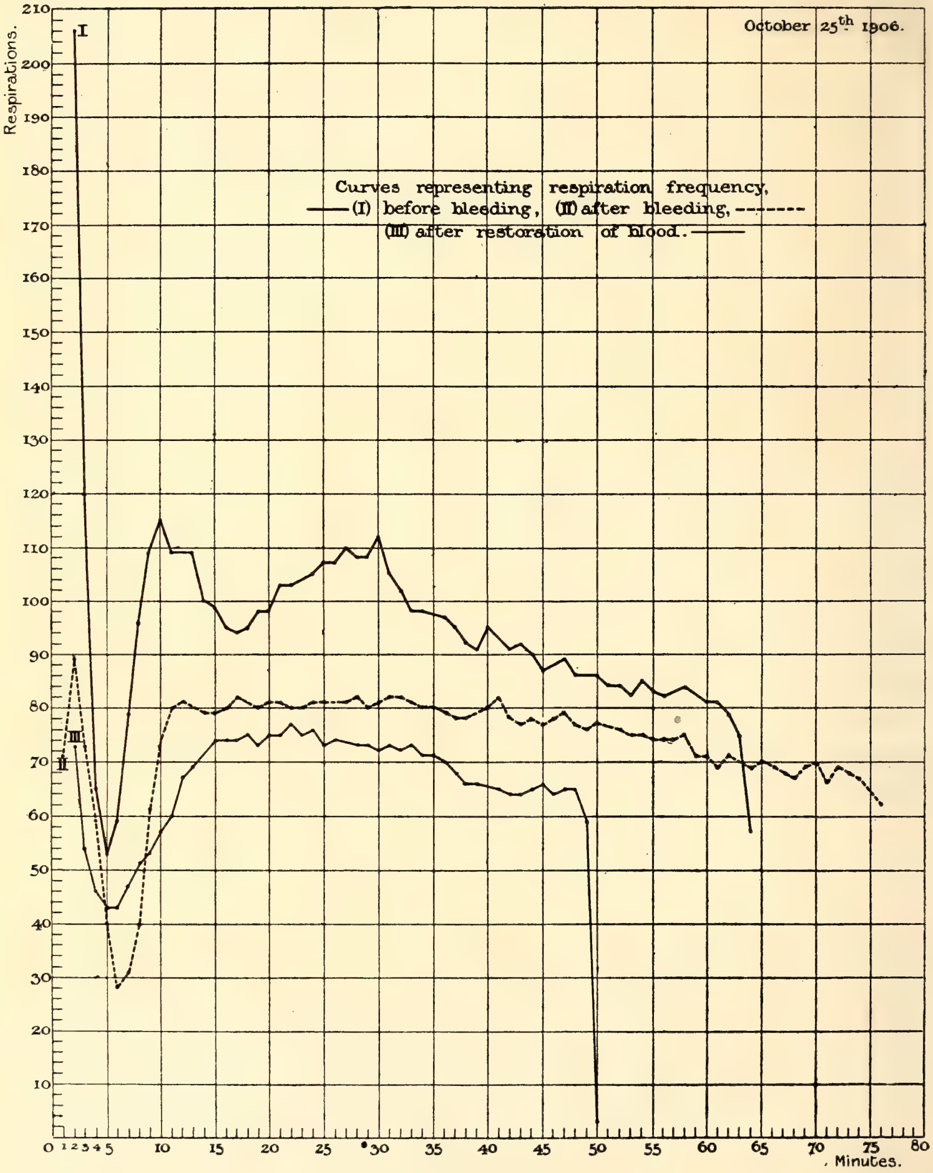
The results given in Table V confirm generally the results of the experiments quoted in the previous tables. Closer results cannot be expected when the prolonged and complex nature of the experiment is taken into consideration. In connection with Experiment XXIV we have thought it advisable to give the tracings of the respiratory movements which were obtained before bleeding the animal, after withdrawal and after replacement of blood, together with curves indicating the rate of the respirations during these three conditions. These tracings and curves are broadly typical of a large number of experiments. In the case of all the experiments in which Woulff's bottle was used, the figures given for the percentage of chloroform administered were obtained by a densimetric estimation at the end of the experiment, and, therefore, only represent the strength of the chloroform-air mixture for the particular time they were taken. They probably indicate, however, within narrow limits, the average value during the experiment.

Conclusions.

The experiments we think show that the percentage of chloroform in the blood does not suffer any variation corresponding to differences in the volume of circulating blood, and the results are therefore in accord with what we should have expected would be the case if the red corpuscles were the essential agents for the transport of chloroform.

As to the duration of time which elapses between the commencement of the inhalation of chloroform and cessation of respiration, it will be noticed that the length of time does not vary according to the volume of blood in circulation; sometimes this was shorter, sometimes longer after than before a hæmorrhage. A somewhat greater equality in time is apparent in those experiments where the respiration ceased at an early period of anæsthesia.

These results are in accordance with the views expressed in a former paper.* We may mention here that a large number of experiments besides those described were made with a view to ascertain this particular point,



EXPERIMENT XXIV.

whether a loss of blood had a direct influence on the duration of anæsthesia, taking as the end-point the cessation of respiration. The results are in agreement with those we have described above, and therefore do not think it necessary to publish them in detail.

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* 'Roy. Soc. Proc.,' *ibid.*

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work. We also desire to express our indebtedness to our assistant, Mr. G. W. Ellis, for the care he has taken in carrying out many of the analyses.

On the Rate of Elimination of Chloroform from the Blood after Anæsthesia.

By George A. Buckmaster, Assistant Professor of Physiology, University College, University of London, and J. A. Gardner, Lecturer on Physiological Chemistry, University of London.

(Communicated by Dr. A. D. Waller, F.R.S. Received July 19, 1907.)

(From the Physiological Laboratory of the University of London.)

In a paper* on the rate of assumption of chloroform by the blood, we showed that the percentage of chloroform rises very rapidly to a maximal value. This is the cause of an early danger-point in anæsthesia. Subsequently a rapid fall takes place, followed by a more or less rapid rise towards a maximal value, which is maintained during the rest of anæsthesia.

The following remarks will illustrate our conception of the anæsthetic process. The blood at first rapidly becomes charged with chloroform, which is held almost entirely by the red corpuscles. The respiratory centre or centres in the cat become affected quite early, and discharge impulses less frequently than the normal. As a result of this the intake of chloroform is lowered, and consequently the percentage of chloroform in blood falls, either owing to the tissues rapidly storing up the drug at the expense of the blood, or because the elimination of chloroform is as rapid or more rapid than the assumption, or to both these causes. If the first danger-point is safely passed, the respirations improve in frequency and become rapid. It is known that many chemical substances, for instance, the group of alcohols, ether or chloroform, which are lethal, primarily act as exciting agents on living cells. Thus those bodies which in a given concentration are lethal for protoplasm, in lower amounts check its activities, but in still less amount, so long as this is above the indifferent point, inversely will act as a stimulus and augment the energy-discharges of protoplasm. In the case of chloroform it would seem that there is an initial stimulating effect which may approach the lethal value, but that if this is not actually attained the stimulating effect, which was masked, persists as a recognisable after-effect when the first danger-point has been passed. Thereafter the chloroform-content of the blood approximates to a maximal value, which is maintained until asphyxia takes place (maximum value).

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* 'Roy. Soc. Proc.,' this volume, p. 555.

If the rate of elimination were more rapid than the rate of intake, or even equal to this, it would not be easy to understand why this maximal value is so rapidly reached or maintained, and for these reasons it appeared important that we should study the rate at which chloroform was eliminated during anæsthesia.

Method.

All the experiments were performed upon cats, which were anæsthetised by ether, and the necessary operations performed after an injection of hirudin into the femoral vein in quantity sufficient to delay the coagulation of the blood during the time of the experiment. After the animal had so far recovered from ether that the reflexes had reappeared but it was still under the influence of the drug, chloroform of about 2 to 3 per cent. was administered from a Woulff's bottle of the form described in our former papers. The inhalation was stopped in some cases when the asphyxial point was reached, and in other cases at an earlier point during the period we have described as the second stage of anæsthesia. Immediately the inhalation of chloroform was stopped, samples of blood were abstracted from the central end of one carotid artery or from the cephalic end of the external jugular vein, or from the side tube of a short T-piece connecting the cephalic and thoracic end of the vein, or by the introduction of a tube several inches in length along the vein, so that a sample of blood could be taken from the right auricle at definite intervals of time. In some experiments only arterial blood was examined, in others only venous, and in other cases samples were taken *simultaneously from both the artery and vein.* The methods of collecting and analysing samples of blood were those fully described in our former papers. In some experiments, however, it was found more convenient to abstract the venous blood from the cannula by means of a syringe. During the entire course of any experiment the respirations were recorded by a tambour placed on the ensiform cartilage.

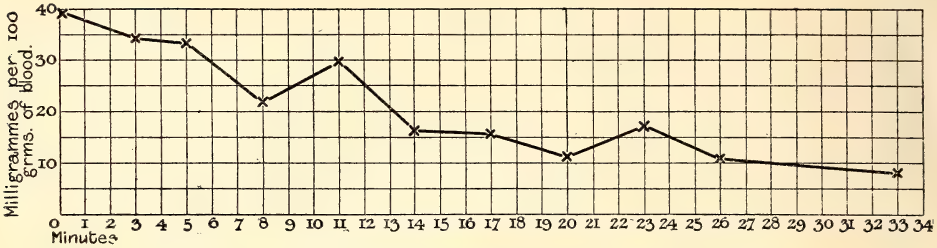
From all our experiments we have selected five typical examples, details of which are given.

Experiment I.

Cat. Weight 3·7 kilogrammes. Respiration recorded by tambour on abdomen. Samples from carotid artery.

Table I.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl in grammes.	Percentage of chlorine in grammes per 100 gr. blood.	Percentage of chloroform.	Colour of blood.	Remarks on respiration.	General remarks.
1.35	Ether on							
2.5	—	3·1586	0·0424	0·3319	—	—		Control sample
2.9	$CHCl_3$ on							
3.14	$CHCl_3$ off	—	—	—	—	—		Breathing feeble; respiration ceased half a minute later
3.14¼	—	3·0899	0·0459	0·3673	0·0397	—	No respirations	
3.15¾	—	—	—			—	Respirations began	
3.17	—	2·147	0·0315	0·3627	0·0346	Bright		
3.19	—	1·9547	0·0286	0·3617	0·0335	Very dark ……	Respirations had become irregular	Respirations ceased at this point
3.20	—	—	—	—	—	—	Respirations began and were regular	
3.21	—	—	—	—	—	—	Cheyne Stokes type	
3.22	—	3·2568	0·0463	0·3515	0·0222	Medium bright		
3.25	—	2·7221	0·0394	0·3578	0·029	Dark		
3.27	—	—	—	—	—	—	Respirations normal and deep	
3.28	—	2·7027	0·0379	0. 3467	0·0166	Quite light …	" " "	
3.31	—	3·3653	0·0471	0·346	0·0159	Very "		
3.32½	—	—	—	—	—	—		Tendon reflex just reappearing
3.33	—	—	—	—	—	—		Left eye reflex
3.34	—	2·5118	0·0348	0·3425	0·0119	Very light ……	Respirations normal to end of experiment	
3.34½	—	—	—	—	—	—		Tendon reflexes were back
3.35½	—	—	—	—	—	—		Ear reflex back
3.36½	—	—	—	—	—	—		Both eye reflexes back
3.37	—	1·9495	0·0274	0·3475	0·0175	Very bright …		Eye reflexes well marked
3.39								
3.40	—	2·9053	0·0402	0·3421	0·0115	" " …		
3.40½	—	—	—	—	—	—		Tail reflexes back
3.45	—	—	—	—	—	—		Pupils normal
3.47	—	2·6158	0·0359	0·3393	0·008	Bright		

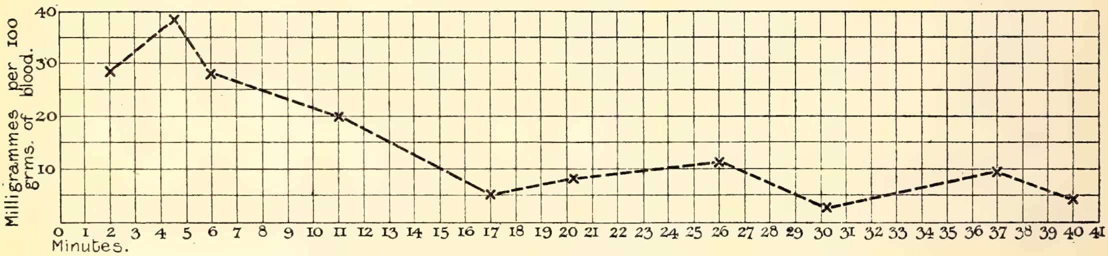


Curve A.—Constructed from Data given in Experiment I.

Ordinates = milligrammes of chloroform in 100 grammes of blood; abscissæ = times in minutes after the cessation of chloroform inhalation; × = samples of arterial blood (eleven).

Experiment II.

Cat. Weight 3·5 kilogrammes. Respiration recorded by tambour on abdomen. Samples taken by a syringe from a long cannula introduced into the external jugular vein, so that blood was withdrawn from the circulation close to the right auricle. It was ascertained *post mortem* that this was the case. The first cubic centimetres of blood withdrawn were rejected so as to avoid taking a sample of stagnant blood in the vein.



Curve B.—Constructed from Data given in Experiment II.

Ordinates = milligrammes of chloroform in 100 grammes of blood; abscissæ = time in minutes after the cessation of chloroform-inhalation; × = samples of venous blood withdrawn close to right auricle.

Experiment III.

Cat. Weight 3 kilogrammes. Samples taken simultaneously from the carotid artery and from the central end of the external jugular vein. The venous blood was withdrawn by a syringe from a cannula pushed down the vein so that the blood was aspirated from the superior vena cava close to the right auricle. The exact position was ascertained *post mortem.* In order to obtain the sample as close to the heart as possible, the blood stagnant in the vein was rejected. The details of this experiment are given in Table III, from which Curve C was constructed.

A number of other experiments were also performed, for example, some in

Table II.

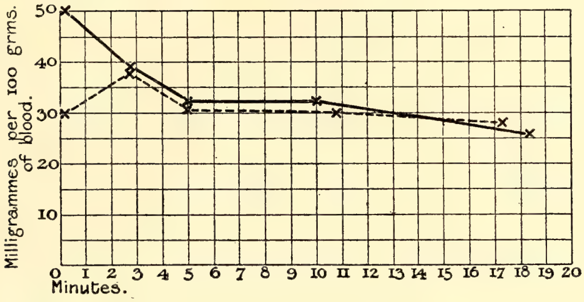
Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl in grammes.	Percentage of chlorine.	Percentage of chloroform.	Colour of blood.	Respiratory remarks.	General remarks.
2.15	Ether started							
2.26	—	—	—	—	—	—		Hirudin injected
2.40	—	2·2176	0·0294	0·32779	—	—		Control sample
2.43	$CHCl_3$ on	—	—	—	—	—		Reflexes well back
3.11	$CHCl_3$ off							
3.11¼	—	—	—	—	—	—	Breathing ceased	
3.12¼	—	—	—	—	—	—	Respirations commence in an irregular way	
3.13	—	2·7634	0·0395	0·3534	0·0287			
3.14	—	—	—	—	—	—	Still irregular; ceased for 25 seconds	
3.15½	—	2·1758	0·0319	0·3625	0·0389	Darkish	Irregular and shallow	
3.16	—	—	—	—	—	—	Respiration normal for this stage	
3.17	—	2·2555	0·0321	0·35187	0·027	Brighter		
3.19	—	—	—	—	—	—		Tube exploded
3.20½	—	—	—	—	—	—		Slight corneal reflex in both eyes
3.21	—	—	—	—	—	—		Tendon reflexes back
3.22	—	1·8378	0·0257	0·3457	0·02	Bright		Corneal reflexes good
3.28	—	3·1199	0·0419	0·33205	0·0048	„	Respiration normal for this stage	
3.31¼	—	2·4824	0·0336	0·33465	0·0077	Rather darker		
3.37	—	2·2745	0·0311	0·33807	0·0115	Dark	„ „	
3.41¼	—	2·3814	0·0318	0·33016	0·0026	Very dark ...	„ „	
3.48	—	2·387	0·0325	0·33663	0·0099			
3.51	—	2·3244	0·0312	0·3319	0·0046	—	„ „	

Table III.

Time.	Anæsthetic.	Artery.					Vein.					Remarks.
		Weight of blood in grammes.	Weight of AgCl in 100 grammes blood.	Percentage of chlorine in 100 grammes.	Percentage of $CHCl_3$ in 100 grammes.	Colour of blood.	Weight of blood in grammes.	Weight of AgCl in 100 grammes blood.	Percentage of chlorine in 100 grammes blood.	Percentage of $CHCl_3$ in 100 grammes blood.	Colour of blood.	
2.45	Ether											
2.56		—	—	—	—	—	—	—	—	—		Hirudin injected in femoral vein
3.14	—	—	—	—	—	—	2·9502	0 0407	0·34109	—	—	Control from vein
3.15	Ether off	—	—	—	—	—	—	—	—	—	—	Artificial respiration
3.23	$CHCl_3$ on											
4.11	$CHCl_3$ off	—	—	—	—	—	—	—	—	—	—	Resp. 53 per min.
4.11¼	—	1·9274	0·0301	0·38612	0·05	—	2·6992	0·0402	0·36823	0·03	—	,, 53 ,,
4.13¾	—	1·1769	0·0269	0 37597	0·039	—	2·6146	0·0397	0·37542	0 0385		
4.16	—	2·2614	0·0338	0·36954	0·032	—	3·2532	0·0485	0·3686	0·031	—	,, 50 ,,
4.21	—	2·9935	0·0448	0·37002	0·0324							
4.21¾	—	—	—	—	—	—	2·7019	0·0402	0·36786	0·03	—	,, 38 ,,
4.24	—	—	—	—	—	—	—	—	—	—	—	Eye and tendon reflexes back
4.28¼	—	—	—	—	—	—	3·6653	0·0543	0·36627	0·028		
4.29¼	—	3·4006	0·0501	0·36426	0·026	—	—	—	—	—	—	Resp. 33 per min.

NOTE.—Respiration uniform in depth throughout the experiment.

which the right external jugular vein was severed and the ends connected by means of a glass tube with a lateral branch from which the blood was withdrawn without interfering with the flow of blood, samples of blood being taken at the same time from the carotid artery. In other experiments, samples were taken simultaneously from the carotid artery and central end of the external jugular vein. In the following Tables IV and V we give the results of two types of these experiments. When the blood was taken without much disturbance to the circulation (Table IV) and was really a sample of particular venous blood coming partly from muscles and partly from the brain, obvious variations in the chloroform-content of the blood occur which may be due to variations in the rate of discharge from the tissues but of the exact significance of which we have no knowledge. Similar variations in the content of venous blood are noticed in the data given in Table V.



Curve C.—Constructed from Data given in Experiment III.

Ordinates = milligrammes of chloroform in 100 grammes of blood; abscissæ = times in minutes after the cessation of chloroform-inhalation; ×——— = arterial blood; ×- - - - - = venous blood.

General Conclusions.

The rate of elimination appears to depend upon the physiological state of the individual animal, but when all the experiments described in this paper are considered generally and compared with those in our previous papers, the rate of output is found to be at first comparatively rapid, and then subsequently becomes slower. The initial rates of elimination are, however, much less rapid than the initial rates of the intake of chloroform, and, therefore, on the whole, elimination is a much slower process than assumption, a view which is borne out by a comparison of the times at which the various reflexes disappear and reappear.

As the results of his experiments on dogs, Nicloux* shows that the arterial blood loses about half its chloroform-content five minutes after inhalation is stopped, and at the end of one hour about one-third to one-quarter of what the blood originally held is eliminated. At the end of

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* 'Comptes Rendus,' 1906, vol. 60, p. 14.

Table IV.

Time.	Anæsthetic.	Artery.					Vein.					Remarks.
		Weight of blood in grammes.	Weight of AgCl in 100 grammes blood.	Percentage of chlorine in 100 grammes.	Percentage of $CHCl_3$ in 100 grammes.	Colour of blood.	Weight of blood in grammes.	Weight of AgCl in 100 grammes blood.	Percentage of chlorine in 100 grammes blood.	Percentage of $CHCl_3$ in 100 grammes blood.	Colour of blood.	
2.15	Ether											
2.52	—	—	—	—	—	—	—	—	—	—	—	Hirudin in femoral vein
2.53	—	3·7841	0·0535	0·34955	—	—	—	—	—	—	—	Control from artery
3.0	$CHCl_3$ on											
3.20	$CHCl_3$ off											
3.20¼	—	3·5269	0·0561	0·39328	0·049							
3.21	—	—	—	—	—	—	2·2695	0·0359	0·39111	0·0466		
3.22¼	—	3·256	0·0502	0·38119	0·0355							
3.22¾	—	—	—	—	—	—	2·1454	0·0338	0·38953	0·045		
3.23¼	—	3·185	0·0487	0·37805	0·032							
3.23¾	—	—	—	—	—	—	2·447	0·0371	0·37486	0·028		
3.24¾	—	3·1778	0·0483	0·37579	0·029							
3.25¼	—	—	—	—	—	—	2·5029	0·0384	0·37933	0·033		
3.26¼	—	3·48	0·0532	0·37797	0·032							
3.26¾	—	—	—	—	—	—	2·5219	0·0392	0·38431	0·039		
3.28	—	—	—	—	—	—	—	—	—	—	—	Corneal reflexes in one eye
3.28½	—	—	—	—	—	—	—	—	—	—	—	Tendon reflex back
3.29	—	—	—	—	—	—	—	—	—	—	—	Both eye reflexes
3.30	—	—	—	—	—	—	—	—	—	—	—	Pupils dilated
3.33½	—	—	—	—	—	—	—	—	—	—	—	Tail reflexes back
3.37	—	—	—	—	—	—	—	—	—	—	—	Eyeballs move; pupils less dilated
3.44	—	—	—	—	—	—	—	—	—	—	—	Eyeballs still rolling
3.45	—	2·9613	0·0439	0·36653	0·019							
3.50	—	—	—	—	—	—	—	—	—	—	—	Not quite round

Table V.

Time.	Anæsthetic.	Artery.					Vein.					Remarks.
		Weight of blood in grammes.	Weight of AgCl in 100 grammes blood.	Percentage of chlorine in 100 grammes.	Percentage of $CHCl_3$ in 100 grammes.	Colour of blood.	Weight of blood in grammes.	Weight of AgCl in 100 grammes blood.	Percentage of chlorine in 100 grammes blood.	Percentage of $CHCl_3$ in 100 grammes blood.	Colour of blood.	
1.35	Ether											
2.40	—	—	—	—	—	—	—	—	—	—	—	Hirudin injected in femoral vein
2.45	—	2·8628	0·0376	0·32473	—	—	—	—	—	—	—	Control
2.58	$CHCl_3$ on											
3.29	$CHCl_3$ off	—	—	—	—	—	—	—	—	—	—	Resp. 36 per min.
3.29¼	—	2·0937	0·0315	0·37198	0·053	Dark	2·07872	0·0306	0·36405	0·044	—	,, 33 ,,
3.31	—	2·9353	0·0432	0·36388	0·044	—	2·0656	0·0304	0·36388	0·044	—	,, 58 ,,
3.32	—	4·1472	0·0609	0·36307	0·043							
3.32¼	—	—	—	—	—	—	1·7525	0·0253	0·35694	0·036		
3.34	—	3·8407	0·0557	0·35957	0·038							
3.35¼	—	—	—	—	—	—	1·4911	0·0217	0·35982	0·039		
3.37	—	3·2474	0·04549	0·34947	0·028	—	—	—	—	—	—	Resp. 58, but shallower
3.37½	—	—	—	—	—	—	2·5604	0·0367	0·35439	0·033	Darkish	
3.39½	—	—	—	—	—	—	—	—	—	—	—	Eye reflexes just back
3.43	—	—	—	—	—	—	—	—	—	—	—	Tendon reflexes back
3.43½	—	—	—	—	—	—	—	—	—	—	—	Tail reflexes back; pupils contracting
3.47	—	—	—	—	—	—	—	—	—	—	—	Pupils normal
3.47¼	—	2·457	0·0345	0·34717	0·025	Bright	—	—	—	—	—	Respiration as last observation

seven hours the blood still may contain 1 to 2 milligrammes per cent. of chloroform. He also observed that the palpebral reflex reappeared within 1½ to 2 minutes after anæsthetic periods which lasted 38 and 67 minutes. In one experiment he mentions that the animal got up and walked off the table 30 minutes after the chloroform was stopped, and the animal appeared to be quite normal, though at this period there were 10 milligrammes of chloroform per cent. in the blood.

In the case of cats, the curves for arterial blood show a general similarity in form to curves which we have constructed from the data given in Nicloux's paper. The initial falls in our curves are not so rapid as in the case of the dog, and the chloroform-content of arterial blood was only reduced by 50 per cent. in about 15 to 20 minutes. In our longest experiments, about three-quarters of the chloroform was eliminated in about 30 minutes, when the animal was breathing normally. When, however, an increased ventilation of the lung is brought about by artificial respiration, a very much shorter space of time is required, as is evident from the analyses quoted in former papers.

Reflexes in our experiments reappear later than was noticed by Nicloux for dogs; but though the pinna reflex, corneal reflex, tendon reflex, and tail reflex were noted, there is no definite order in which they reappear, though as a rule the tail reflex is the last.

Not infrequently an animal goes to sleep during recovery from anæsthesia, but we have never noticed the rapid recovery of volition to which Nicloux has drawn attention.

According to Tissot's observations, it would appear that during recovery from anæsthesia the amount of chloroform in venous blood constantly exceeds the amount in arterial, as may be seen from the following table constructed from his paper. He also considers that a study of the chloroform-content of arterial blood should be made during the induction of anæsthesia and of venous blood during the disappearance of anæsthesia.

	Cessation of chloroform administration.	45 minutes later.	2 hours later.
Arterial blood ...	53·2	5·8	0·0
Venous blood ...	48·1	7·7	4·9

All these results we cannot confirm, though in Tables III, IV, and V, which are those of the chloroform-content of samples of arterial and venous blood taken simultaneously, we are in agreement with Tissot's observations that at the moment when chloroform is stopped, arterial blood contains an excess of the drug when compared with venous blood.

For the sake of argument, we will assume that during recovery from anæsthesia the blood circulates in the body, and that no exchange of chloroform takes place between the blood and tissues. Under such conditions it is clear that, given a steady elimination at the surface of the lungs, the amount of chloroform in arterial blood would be constantly less than in venous, and this difference would probably be a decreasing one. During the initial rapid elimination, this difference would conceivably be marked and easily detectable by experiment; but in the later stages, where the elimination is slow, the difference would possibly lie within the errors of experiment. But during recovery from anæsthesia there will be superimposed on this simple conception of the circulation a discharge of chloroform from the tissues into the venous blood. If this took place steadily, it would tend to increase the difference between arterial and venous blood. The problem, however, appears to be more complex. First of all, the blood comes from regions such as the muscles, viscera, etc., which are probably not charged to any great extent with chloroform, compared to what is believed to be the case for the central nervous system. The nerve centres are differently affected, and doubtless discharge at different rates, as the evidences of their activity or paralysis appear and disappear irregularly. Further, it is not inconceivable that during the elimination one region may be discharging chloroform while other cell-districts are relatively inactive in this respect, or even actually continuing to absorb chloroform. The question will be further complicated in a very marked degree by the nature of the respiration at any moment, for the depth and frequency of respirations naturally govern the elimination at any particular moment. On the whole, therefore, we should expect the chloroform-content of venous blood to be higher than that of the arterial, but this difference would be slight in the later periods of elimination. In actual experiments one would expect the result would depend markedly on the particular source whence the venous blood was taken. A comparison with Curves A and B shows a general similarity in the rate of elimination both in the case of arterial and venous blood. Both curves show irregularities in the rate. In Curve C, the chloroform-content of arterial blood is contrasted with the general venous blood of the body (sample taken close to the heart). At the moment of cessation of respiration, the arterial blood is markedly higher than the venous, but after regular respiration is established, the two curves are practically identical, the difference being within the errors of experiment.

The work has been carried out with the help of a grant which was made to us by the Government Grant Committee of the Royal Society, for which we now express our thanks.

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